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DEVELOPMENTAL ABNORMALITIES OF THE EYE

By

IDA C. MANN

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With a Foreword by

SIR JOHN H. PARSONS

Published for the *British Journal of Ophthalmology*

This book is the logical successor to Miss Mann's earlier book "The Development of the Human Eye." It applies a knowledge of embryology to the elucidation of clinical problems, and is mainly, though not entirely, based on Miss Mann's practical experience. Each abnormality is described and pictorially illustrated, and its pathogenesis fully discussed. Every reasonable hypothesis is cited and critically examined, but the author has not refrained from definitely stating her own opinions.

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THE PRIMITIVE FORM OF EPIPHYSIS IN THE LONG BONES OF TETRAPODS

By R. WHEELER HAINES

Department of Anatomy, St Thomas's Hospital Medical School

The earlier work on the evolution of epiphyses was directed for the most part towards the question of the presence and function of secondary centres of ossification or calcification in the cartilages at the ends of long bones, and the word epiphysis was usually confined to these secondary centres (Parsons, 1905; Moodie, 1908). An extended study of the microscopical structure of the more primitive vertebrates has, however, directed attention to the arrangement of the cartilage itself, rather than to the secondary centres which may be developed in it, for the fundamental mechanisms of growth in the cartilage now seem to be similar in all classes of bony vertebrates, while secondary centres occur constantly in mammals, but only sporadically in reptiles, amphibians and fishes. Further it now appears that one of the functions, if not the most important function, of an epiphysial formation is the determination of the arrangement of the trabeculae in the endochondral bone laid down in relation to it. So the use of the word epiphysis has become extended, and will here be used to include the whole of the cartilage between the articular surface of the bone and the region of endochondral ossification, whether this contains a secondary centre or not.

The structure of epiphyses and of the endochondral bone related to them has now been described and figured in several groups of vertebrates. Following the early work of Gegenbaur, Eggeling (1911) attempted to reach an understanding of the evolutionary sequence of the forms encountered in different animals by an intensive study of the urodele amphibians, and this was followed by Lubosch (1924) in birds, Froböse (1927) in anurans, and Heidsieck (1928) in certain groups of lizards. In urodeles the epiphyses show a gradation from the simple types found in the phanerobranchs such as *Proteus*, in which the cartilage in the axis of the long bones may persist throughout life and there is no endochondral bone, through cryptobranch types in which the cartilage is eroded by several small distinct marrow cavities, each with a partial wall of endochondral bone, to types found in the caducibranchs, in which a large single marrow cavity occupies the whole of the shaft so as to divide the cartilage completely, and may even project beyond the shaft towards the articular surface.

Now Eggeling decided that the simpler types found in phanerobranchs and cryptobranchs were derived phylogenetically from the more advanced types by a neotenic process, since the early fossil amphibians already possessed

well-developed marrow cavities. But in the absence of other evidence it seemed probable that the tetrapods had, since their evolution from fishes, followed in their development the stages shown by the neotenic urodeles, and later authors have been accustomed to refer to the urodeles as illustrations of the primitive tetrapod type. It is now known, however, that epiphysial structures and endochondral bones occur constantly in typical bony fishes (Haines, 1934), so that it appears probable that the early tetrapods inherited a relatively advanced form of epiphysis from their piscine ancestors, and the structures found in other groups should therefore be compared with those in such a primitive stock rather than with those in modern urodeles. Here an attempt will be made to define the primitive form of epiphysis in tetrapods as closely as possible by a study of living fishes, of living tetrapods which appear to have retained the primitive epiphysial structure, and of such fossil material as is available.

It would of course be of the greatest interest to know something of the early evolution leading to the formation of epiphyses. But these are already fully formed in fishes, and though a search has led to the discovery of some new and extraordinary arrangements of cartilage and bone (Haines, 1937) nothing can be said of the evolution of epiphyses themselves.

EPIPHYSES IN FISHES

The structure of epiphyses in fishes has been described in detail by Haines (1934). Two further illustrations are given here for comparison, one a small branchial from the primitive teleost *Elops saurus* (Fig. 1), and the other a radial from the pectoral fin of a gurnard (Fig. 2).

In the branchial bone a large mass of cartilage with undifferentiated cells (*z.u.c.*) projects far beyond the shaft, in the radial not so far, and in both cases it is covered by a thick layer of articular fibro-cartilage (*a.f.c.*). Opposite the end of the shaft a distinct growth zone is formed by a layer of cells which are flattened (*z.f.c.*) but have no regular arrangement. Derived from these cells are the hypertrophied cells (*z.h.c.*); these are large and rounded and are scattered through the matrix, which may be calcified (*c.z.h.*). The shaft of periosteal bone (*p.s.*) surrounds the base of the epiphysis as far as the zone of flattened cells. Within the shaft the amount of erosion of the hypertrophied cartilage is very variable, for sometimes the cartilage is entirely unresorbed, sometimes it is resorbed only in the centre of the shaft, and sometimes, as in the specimens illustrated, the surface of erosion approaches the growth zone, leaving only a narrow layer of hypertrophied cells.

In the marrow cavity endochondral bone (*e.b.*) is usually plentiful but has no regular arrangement, though transverse trabeculae (*t.t.*) can be recognized at intervals in some bones. These are laid down originally on the eroded surface of the hypertrophied cartilage, and later isolated by the destruction of the cartilage beyond it, so that they come to lie freely in the marrow cavity. Some

of the trabeculae may contain isolated remnants of hypertrophied cartilage (*i.h.c.*), forming structures comparable to the primary trabeculae of other animals.

In other fishes the essential structure is the same. Lubosch (1910) has figured sections of the fins of several fishes showing endochondral bone formation (*Amia, Tinca, Leuciscus, Clupea*) and it may be taken that the structure is primitive for bony fishes as a whole, and that the early tetrapods probably inherited this structure. The continuous core of unresorbed cartilage found for

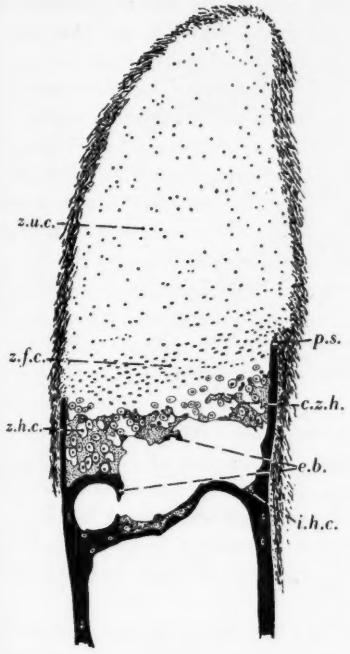

Fig. 1. Longitudinal section of the lower end of the hyobranchial of *Elops saurus*.

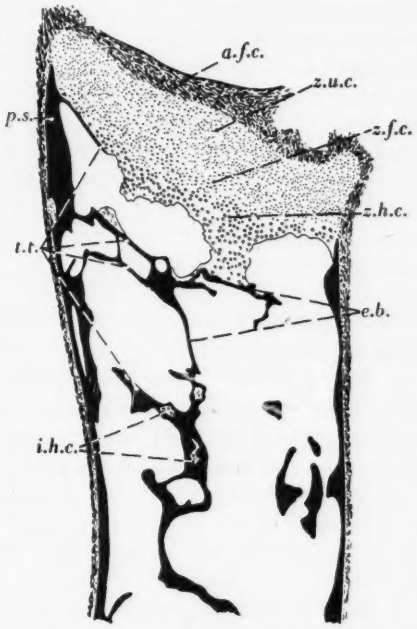

Fig. 2. Longitudinal section of the inner end of a radial of *Trigla*.

instance in modern Dipnoi is a specialization. In *Sciaena*, a specialized teleost, a secondary centre of calcification has been found in the zone of undifferentiated cells, a remarkable parallel to the development of such centres in other groups (Haines, 1934).

EPIPHYSES IN CHELONIANS

Tetrapods can be grouped into those whose epiphyses remain cartilaginous throughout life, and those possessing secondary centres of calcification or ossification. The first group includes all fossil land animals from the earliest carboniferous to at least Triassic times, and is undoubtedly primitive, while

the second includes the vast majority of living animals, the only exceptions being the chelonians, crocodiles, urodeles and some birds. Now it will be shown later that the birds appear once to have had secondary centres and to have lost them, and that the epiphyses of urodeles are highly specialized structures very different from the primitive form. This leaves only the chelonians and crocodiles as possible living representatives of the primitive type.

In a newly hatched *Homopus areolatus* (Fig. 3), taken as a type, the articular surface is carried by a large mass of undifferentiated cartilage (*z.u.c.*) which projects far beyond the end of the shaft, and in the case of the femur overhangs it ventrally. In the zone of growth (*z.f.c.*) the cells are flattened and are more crowded than in the undifferentiated cartilage, but are not yet arranged in longitudinal clumps or columns. The primary marrow cavity (*p.m.c.*) in the centre of the shaft has not progressed far towards the ends of the bone, so that the zone of hypertrophied cells (*z.h.c.*) forms a long regular cone of cartilage projecting far into the shaft and anchoring the whole epiphysis to the periosteal bone. The parts of the hypertrophied cartilage which were formed first, those towards the centre of the shaft, are uncalcified, but the older parts show an irregular area of calcification of the matrix next the perichondral bone (*p.a.c.c.*), and near the growth zone the calcification spreads inwards and a few small areas are found in the centre of the shaft (*c.a.c.c.*). The main surface of erosion (*s.e.*) of the hypertrophied cartilage, facing the primary marrow cavity, is smooth, and no endochondral bone is laid down on it. But besides the main cavity several accessory marrow cavities (*a.m.c.*) are eroded in the peripheral calcified matrix by lateral marrow buds which perforate the bony shaft independently, and small plates of endochondral bone (*e.b.*) are laid down on the eroded surfaces. The periosteal bony shaft (*p.s.*) is already strengthened by accessory plates of periosteal bone (*a.p.p.b.*), but there is as yet no evidence of secondary resorption of bony substance, except where the marrow sprouts have broken through the bone to reach the cartilage.

In an older example of the same species (Fig. 4) the epiphysial structure is much further advanced. In the young animal the growth zone forms a relatively flat plate of cartilage lying at the level of the end of the bony shaft, but this zone (*z.f.c.*) is now strongly curved so as to lie nearer the articular surface, while the zone of undifferentiated cells (*z.u.c.*) is relatively reduced. The change in shape of the growth zone has been brought about by an addition to the convex surface of the growth zone from the deeper lying undifferentiated cells, and a corresponding reduction on the concave surface by a hastening of the processes of hypertrophy, so that the zone as a whole is carried outwards to the matrix. The cells of the growth zone are more sharply marked off than before, and the hypertrophied cells are in places arranged in loose columns which lie in the direction of the longitudinal axis of the bone (*c.clm.*). Near the periphery the columns are parallel to the bone forming the end of the periosteal tube, and so lie obliquely to the plane of the growth cartilage

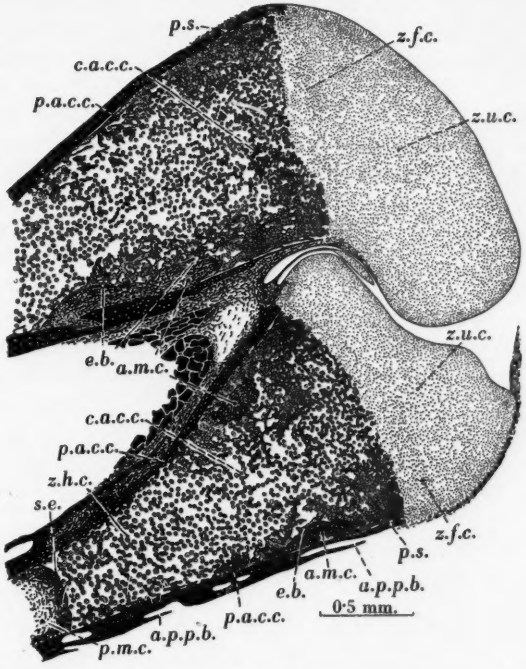

Fig. 3. Longitudinal section of the knee of a newly hatched *Homopus areolatus*. The bones have been forced into an unnatural position for convenience of sectioning.

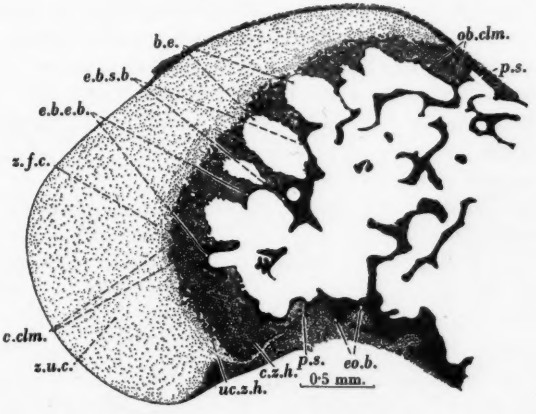

Fig. 4. Longitudinal section of the lower end of the femur of a half-grown *Homopus areolatus* (*Carapace length* 6·1 cm., adult length 10 cm.).

(*ob.clm.*), not at right angles to it as in the more central parts of the shaft. The hypertrophied cartilage is now fully calcified (*c.z.h.*), leaving only a narrow layer of uncalcified cartilage with hypertrophied cells just below the growth zone (*uc.z.h.*).

The primary and accessory marrow cavities have now fused, and the hypertrophied cartilage is reduced to a relatively narrow layer which follows the shape of the growth cartilage. The advancing surface of the marrow, instead of lying flat as before, is now developed into a series of processes, each of which erodes an elongated bay (*b.e.*) in the hypertrophied cartilage, sometimes progressing as far as the uncalcified layer. The bays follow the direction of the cell columns, by which they seem to be guided, each one corresponding in thickness to several cartilage cells, and each separated from its neighbours by a wall of cartilage containing many cells. On the walls of the bays, both on their sides and at their ends, endochondral bone is laid down (*e.b.s.b.* and *e.b.e.b.*), and when the cartilage is removed the bone so determined persists as the trabecular system. Secondary alteration of the periosteal bone is now seen clearly on the ventral surface of the shaft, where the primary sheath (*p.s.*) is eroded soon after its formation, and is replaced by a thick mass of new bone of endosteal origin (*eo.b.*).

In a sexually mature *Testudo pardalis* the hyaline cartilage at the lower end of the femur had been still further reduced by the advance of the zone of growth towards the articular surface, but it still formed a thick pad of tissue over the end of the bone. In the upper end of the radius the zone of undifferentiated cells was reduced to a narrow layer beneath the articular cartilage, and the zone of flattened cells could no longer be recognized, but the bone structure was similar to that in the younger specimen.

So far as is known, all chelonians conform to the one type, but no early stages are known from other species. Wallis (1927) has published a valuable description of the cellular arrangement in *Clemys leprosa* at a stage corresponding to my Fig. 4 or a little older. The cells of the growth zone were more definitely clumped and the hypertrophied cells remained uncalcified rather longer, otherwise the structure was similar. Adult structure is illustrated by Moodie (1908) in cleared preparations, and in more detail by Lubosch (1910) in *Emys* and *Chelydra*, and is similar throughout.

The replacement of the matrix by endochondral bone may be further studied from models of thick sections (Fig. 5). Each marrow process, shown in the model as a hollow in the matrix, begins as a blunt rounded extremity (*a.b.e.*), where the marrow is usually eroding the cartilage actively. The side walls of the cavities may be parallel (*p.w.b.e.*), indicating that erosion is quiescent, or they may diverge (*d.w.b.e.*), indicating that erosion is still widening the cavity. At the bases the cavities open into the general spaces of the bone. The bays are evenly distributed over the whole area of erosion, and are of approximately equal size, so that the area for the head of the femur has seven or eight in each section, and the area for the trochanter only one or two.

The endochondral bone laid down on the surface of the cartilage is partly removed by secondary erosion, and what remains is modified so as to leave a fairly close meshwork of trabeculae filling in the interior of the bone. But the trabeculae which persist still retain their first arrangement, so that they consist chiefly of longitudinal plates (Fig. 5, *l.t.*) straight in longitudinal section but curved in the transverse plane, as were the side walls of the marrow cavities on which they were deposited, and, just like the cavities, these trabeculae radiate towards the growth cartilage. Occasional transverse trabeculae (*t.t.*), laid down at the extremities of the marrow processes, can be found. In

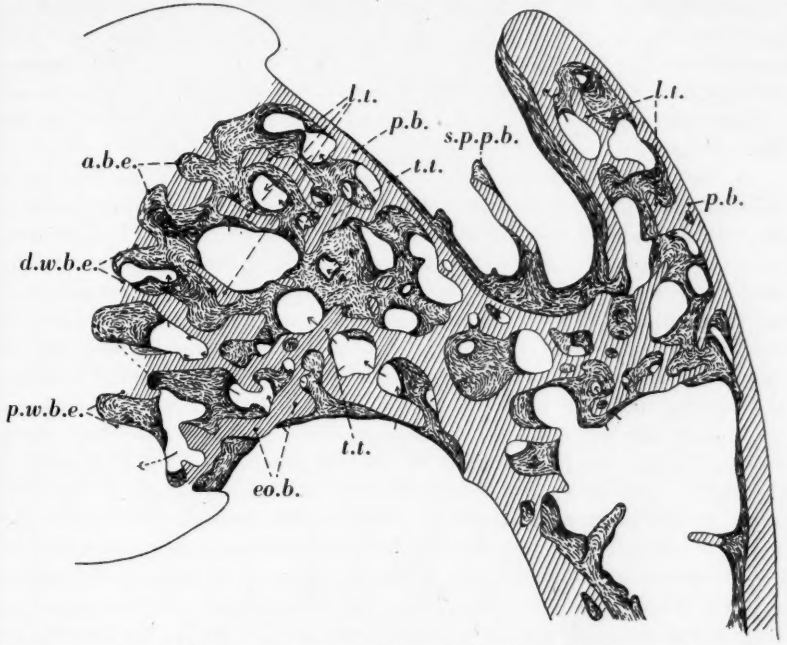

Fig. 5. Reconstruction in blotting-paper and wax of a thick section from the upper end of a femur of a half-grown *Homopus areolatus*.

single sections it is difficult to distinguish an obliquely cut longitudinal plate from a true transverse plate, but in models the distinction is clear. Such plates are usually perforated by secondary resorption. The texture of the endochondral bone is uniform throughout its extent, thus differing from mammalian and other types in which it changes markedly at different levels below the growth cartilage. The shaft below the head is deeply undercut to form the head, the place of the periosteal bone being taken by endochondral trabeculae thickened by endosteal action (*eo.b.*). The rest of the neck and trochanter are covered by periosteal bone (*p.b.*), and in the gap between them large secondary bony processes (*s.p.p.b.*) grow from it into the connective tissue.

The radiate arrangement of the trabeculae, not yet very clear at the upper end of the femur and less so at the lower end, becomes more marked in older bones, where it is evident on naked eye inspection. At no time is there any evidence of an extensive remodelling of the trabecular system in response to mechanical or other factors.

EPIPHYSES IN CROCODILES

In a newly hatched crocodile (Figs. 6 and 7) the structure of the epiphyses resembles that of the half-grown tortoise already described. It cannot be stated with certainty that a deep cartilage cone eroded by both primary and accessory marrow cavities was formed at an earlier stage, but this appears probable from the occurrence in several sections of both the tibia and the femur of large masses of hypertrophied cartilage now completely isolated in the marrow cavity.

In the present stage the growth zone (*z.f.c.*), in which the flattened cells are arranged in columns of some regularity, has already become convex towards the articular surface and lies roughly parallel to it. The zone of hypertrophy (*z.h.c.*) is comparatively wide and the cells form long irregular columns. The marrow processes (*m.p.*) are more elongated than those of the tortoise, and end on an even plane some distance below the growth cartilage towards which they radiate. The phases of erosive activity of the marrow can be distinguished clearly in the crocodile. All the processes appear to be eroding the cartilage at their tips, which are nowhere covered with bone (*a.b.e.*). Nearer their bases bony plates are laid down on the remains of the cartilage, and these plates necessarily take the form of more or less complete bony tubes embedded in the matrix, open at one end towards the tips of the cavities, and at the other to the general marrow cavity (*tb.e.b.*). This regular arrangement is broken by a second zone of erosion in which the remains of the cartilage and much of the bone is destroyed, so as to form a general marrow cavity with a wide meshed network of bone. The trabeculae, as seen in the model, have a predominantly longitudinal radiating arrangement (*l.t.*), but this, owing to the immaturity of the bone, is not yet well marked. As in the tortoise, adult bones demonstrate the radiate arrangement more clearly.

The precise changes can be followed in more detail in a series of transverse sections from another tibia (Fig. 8, A–D). The first passes through the tips of the processes before they have reached their full diameters. The cartilage, whose matrix is calcified, contains hypertrophied cells of the usual type, which show however no marked degenerative changes. The walls of the cavities are irregular, having small pits obviously derived from the destruction of one wall of the capsule of a hypertrophied cell (*h.c.p.*), and in some what is apparently the old cell body is still to be found (*h.c.b.*). Between the pits spicules of cartilage matrix are left standing (*s.c.m.*). This is the well-known appearance of active erosion of cartilage.

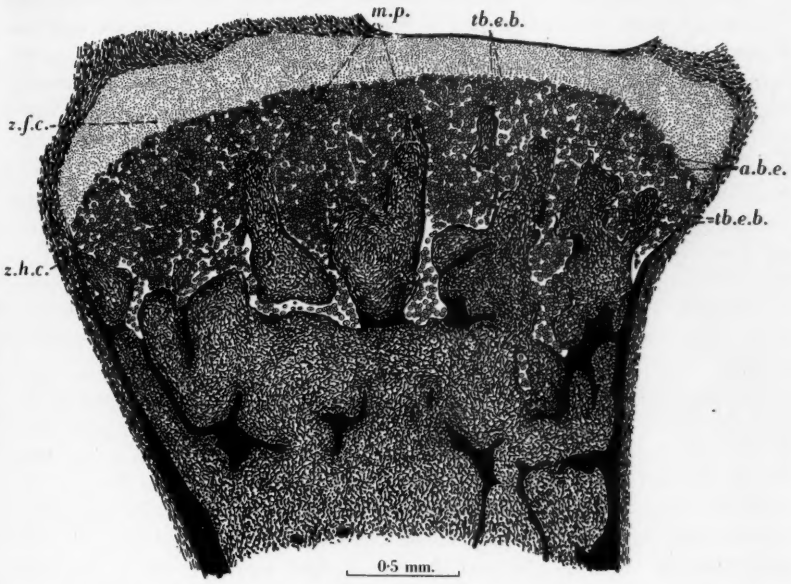

Fig. 6. Longitudinal section of the upper end of the tibia of a newly hatched *Crocodilus niloticus*

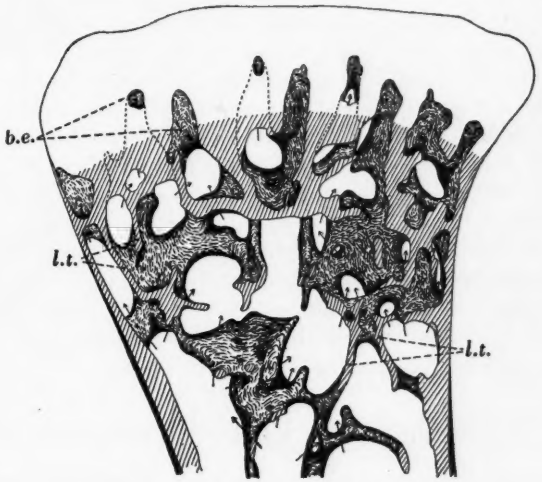

Fig. 7. Reconstruction in blotting-paper and wax of a thick section from the upper end of the tibia of a newly hatched *Crocodilus niloticus*.

In the next section the marrow processes are wider, but are still separated by massive walls of cartilage four or five cells deep. Bony plates are now laid down on the walls of the processes, taking their curved shape, so as to form the series of tubes already described. The surfaces next the marrow are smooth and lined with a layer of osteoblasts (*o.b.*). The convex surfaces are covered with rounded bosses of bone (*g.o.*) connected to the main plates sometimes by a broad base, and sometimes only by a narrow stalk (*s.g.o.*), and some of these bosses contain one or more cells (*c.g.o.*) resembling the undegenerated hypertrophied cells of the cartilage. These "globuli ossei" were described by Kassowitz (1881) in a similar position in young human material, and have been fully discussed recently by Weidenreich (1930). There is still much doubt whether the cells contained in them come from the cartilage or the marrow. Where the cartilage is still exposed to the marrow erosion proceeds as before.

In section C, from the zone of secondary erosion, the predominant feature is the removal of most of the remaining cartilage, which where it is exposed to the marrow shows evidence of very rapid destruction (*s.c.m.*). The bone which was laid down around the marrow processes comes to lie freely in the marrow cavity as a series of arcs and circles, whose outer surfaces, where they are exposed to the marrow, become surrounded by osteoblasts (*o.b.*) and smoothed off. Not all the cartilage is resorbed however, for in the older trabeculae (section D) remnants of the matrix (*c.m.*) still form a core to the bone, and globuli ossei (*g.o.*) varying from a thin shell of bone surrounding a large cell to a boss of solid bone are very conspicuous. Even towards the centre of the shaft the trabeculae still contain some remnants of cartilage matrix. Thus there can be no complete resorption of the primary trabeculae and replacement by a secondary system in the crocodile, for if there were then all the trabeculae would be made of solid bone without cartilaginous inclusions. If, as has been claimed by Schaffer (1888), this secondary substitution occurs regularly in mammals, its absence in primitive reptiles constitutes an important point of difference between the two groups.

References to crocodilian structure are rare. Lubosch (1910) figures a fourth metatarsal and phalanges from a full-grown *Alligator sclerops* which, apart from the difference in size, agrees well with my description. The endochondral bone ends sharply along the curved surface which follows the shape of the articular surface, and the trabeculae radiate towards this surface. Vialleton (1919) mentions the width of the cartilaginous walls between the marrow processes, but gives no figures.

EPIPHYSES IN EARLY TETRAPODS

The cartilaginous parts of epiphyses are not preserved in fossils, but the structure of the bone is sometimes visible on broken surfaces, and can be studied in detail in polished sections. No data concerning the arrangement of

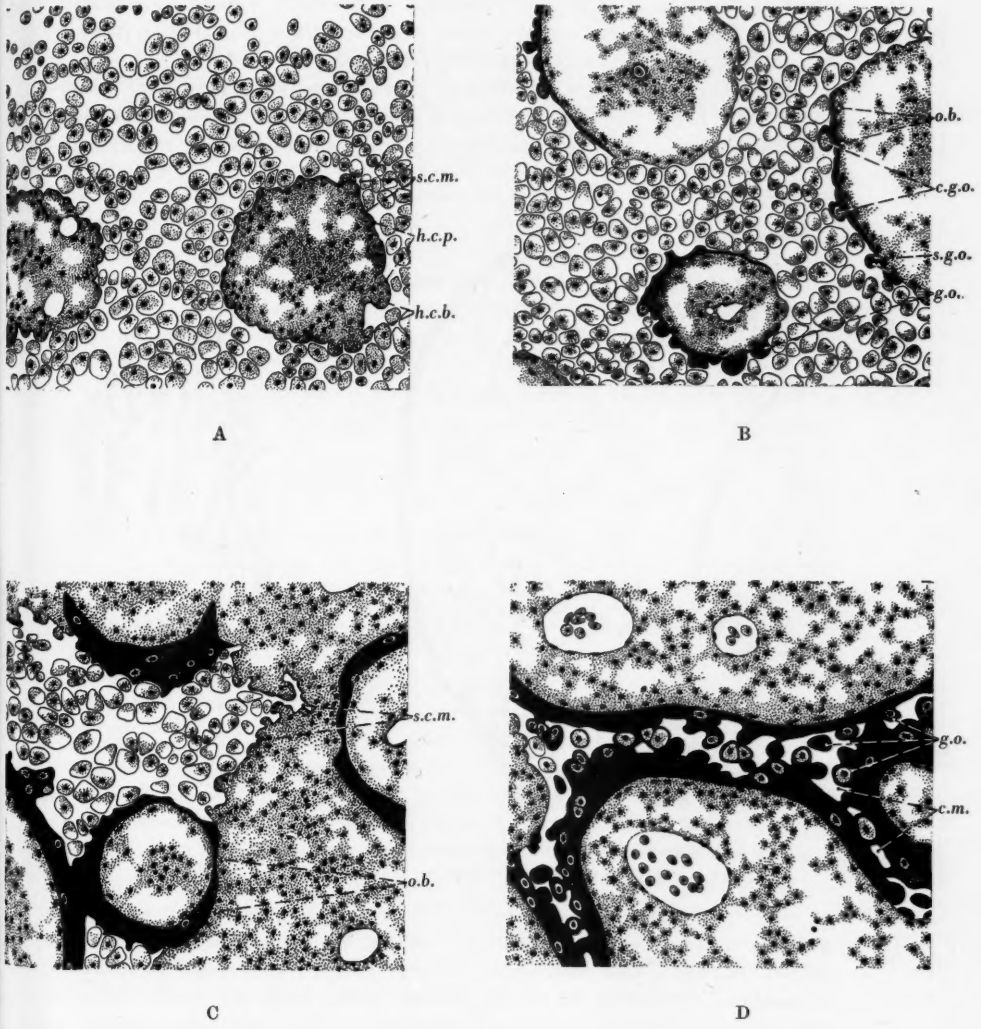

Fig. 8 (A–D). Transverse sections from the upper end of the tibia of a newly hatched *Crocodilus niloticus*.

the trabeculae in primitive groups are available in the literature, but a study of the fossil material at the British Museum has shown that the radiate arrangement of the trabeculae is truly primitive. This is very well demonstrated in a section from the upper end of a femur of a small *Dicynodon* (Fig. 9) in which the endochondral bone was beautifully preserved.

The articular cartilage is carried by a smooth well-ossified convex surface (*art.s.*), and below the head the shaft is slightly undercut (*a.unc.*). The longitudinal trabeculae are well marked (*l.t.*), and radiate towards the articular

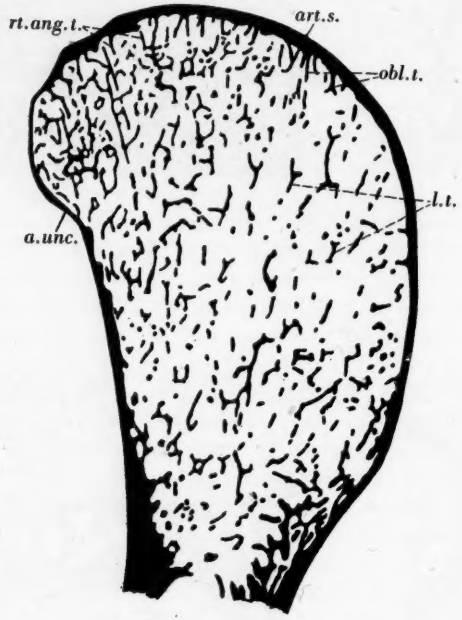

Fig. 9. Longitudinal polished section of the upper end of the femur of a small *Dicynodon*.

Fig. 10. Endochondral bone on the broken surface of an amphibian from Aust cliff (British Mus., R. 299).

surface, those near the centre of the convexity striking the surface at right angles (*rt.ang.t.*), and those near the periphery striking it obliquely (*obl.t.*), a condition paralleled in the lower end of the chelonian femur (Fig. 4). Similarly on the broken surface of a fragment of an amphibian long bone from the Aust cliff Rhaetic formation (Fig. 10), preserved in the British Museum (R. 299), the trabeculae are clearly arranged in radiate fashion.

Now it has been seen that this radiate arrangement depends on the shape of the growth cartilage, and the disposition of the cell columns in the hypertrophied cartilage, which guide the growth of the marrow processes, so that it is

reasonable to suppose that these animals possessed an epiphysial mechanism similar to that of modern crocodiles and chelonians. The trabecular arrangement in all other living groups of tetrapods has lost the primitive radiate arrangement.

The microscopic structure of fossil bones has been studied by Hasse (1878). In Plesiosaurus and Nothosaurus the calcified cartilage with well-marked spaces for the hypertrophied cells can be recognized forming wide areas between the bony lamellae, so that the whole characteristic structure can be compared with that in the crocodile (Fig. 8 B, C). But in the fossil animals the cartilage persists in the adult, whereas in the crocodile it is mostly destroyed soon after its formation. This, however, is probably a specialization peculiar to these secondarily aquatic animals, not a primitive feature.

Secondary centres are never found in the bones of early tetrapods. The earliest form recorded as possessing them is the Jurassic Sapheosaurus, related to the modern *Sphenodon* which has similar secondary centres (Meyer, 1859; Fuchs, 1908).

Whether the earliest tetrapods had their bones as thoroughly ossified as *Dicynodon* or modern chelonians is doubtful. The embolomerous amphibians of carboniferous times, the most primitive known group, had bones with expanded ends carrying the articular cartilages (*Eogyrinus* and *Diplovertebron*, Watson, 1926; *Cricotus*, Romer, 1922), but judging from the shape of the supporting endochondral bone the cartilage appears to have been relatively thick. This feature is also well shown by a perfectly preserved femur in the British Museum (R. 5601, Swinton, 1936) from a carboniferous amphibian, and by the femur of another carboniferous animal Papposaurus (R. 4805, Watson, 1914*a*). In the later rachitomous amphibians, a more advanced grade thoroughly adapted for walking on hard ground, the bones were very thoroughly ossified (*Eryops*, Miner, 1925; *Acheloma*, Romer, 1922). Again in *Seymouria* (Watson, 1918), the most primitive known reptile, there was a large mass of unossified cartilage at the end of the shaft, though this may be due to the immaturity of the best known material. Similarly the bones of Eunotosaurus, possibly an ancestral chelonian (Watson, 1914*b*), were relatively poorly ossified. It appears then that though in the earliest tetrapods the cartilage was reduced as compared with fishes, the final stages in the reduction were carried out independently in different groups, probably in response to a mechanical demand for a rigid structure.

Again the bones of early tetrapods were on the whole more massively built than those of modern animals, and the evolution from a short thick bone to a long slender structure has occurred repeatedly in different groups. Also the articular surfaces were in primitive animals carried on the actual ends of the shafts, not on the necks carved out by the undercutting of the bone, and here again parallel evolutions have occurred. But the actual form of the epiphyses seems always to be similar to that of modern crocodiles and chelonians.

Summing up for comparison the epiphysial characters of fishes and tetrapods: in fishes there is a large mass of cartilage beyond the shaft, the growth zone forms a flat layer opposite the open end of the shaft and the cells of this layer are scattered irregularly through the matrix, the cells of the hypertrophied cartilage are also irregularly scattered and the matrix is often poorly calcified, the marrow processes are rounded, and the endochondral bone forms a network of trabeculae with no structural arrangement. In primitive reptiles and amphibians on the other hand the cartilage is reduced to a relatively narrow layer below the articular surface, the growth cartilage is bulged as a convex sheet beyond the level of the shaft, the cells of the growth zone are arranged in vertical clumps or columns which radiate towards the articular surface, the hypertrophied cells have a similar radiate arrangement, and this is followed by the elongated marrow processes and by the endochondral bone which is laid down as a series of tubes lining the walls of the marrow spaces.

All these changes can be accounted for as leading to a strengthening of the skeletal supports during the evolution of the ancestral fishes into the heavy-bodied early tetrapods. The reduction of the undifferentiated cartilage and the heavy calcification of the hypertrophied region made the ends of the bones firmer. The convexity of the growth zone was a necessary secondary effect of the reduction of the cartilage. The changes in the cellular arrangement of the cartilage leading to a regular longitudinal disposition of the trabeculae would also increase the mechanical efficiency of the bone, but the radiate structure was probably a secondary effect dependent on the convexity of the growth zone. It will be seen later that when land animals become secondarily aquatic, the epiphyses and the endochondral bone tend to return to a condition similar to that found in fishes.

It is probable that the formation at one stage in development of extensive cartilage cones, and their destruction by accessory marrow cavities, are also primitive tetrapod features, and that they are dependent on mechanical demands. The cones are described here for the first time in chelonians and doubtfully in crocodiles, and they are well known in birds, though here the destruction is carried out by a ring of long marrow processes which lie in the cartilage just deep to the shaft and not by accessory marrow cavities. In birds the cones clearly serve to anchor the overlying cartilage to the shaft (Lubosch, 1924). In typical fishes such structures are not conspicuous and secondary cavities do not occur. It seems probable therefore that the cones were specially developed in tetrapods as anchoring structures, and that the formation of secondary marrow cavities and of endochondral bone first at the periphery only, and later near the axis of the bone, allowed for a gradual change from the cartilaginous to the bony anchorage, with no interference with the strength of the bone while the change occurred.

SPECIALIZATION OF EPIPHYSIAL STRUCTURES

The primitive radiate arrangement of the trabeculae was an advance on the irregular arrangement in fishes, but was far from being the most efficient arrangement possible, and further evolution appears to have been directed towards its greater perfection. The primary trabeculae necessarily radiate towards the growth cartilage, and if this lies next to the articular cartilage the arrangement of the trabeculae is then determined by the shape of the articular surface. So long as this surface is situated at the end of the shaft the arrangement of the trabeculae is well suited to the forces they have to withstand, but when the articular surface is carried away from the line of the shaft by a well developed neck of bone conditions are altered. A comparison between the trabeculae in the neck of the femur of the tortoise (Fig. 5) and a man brings out clearly the difference between the simple radiate arrangement and a developed trajectorial system.

One of the factors responsible for the change was the separation of the articular and growth cartilages, so that the growth cartilage could take up any shape convenient for the determination of an efficient system of trabeculae. The separation had existed before in fishes, but in land animals, where firmness is essential, the intermediate cartilage is strengthened by a secondary centre of calcification or ossification. Parallel evolution has produced a similar result in at least five lines of land animals, amphibians, *Sphenodon* (Lubosch, 1910), lacertilians (Heidsieck, 1928) birds and mammals. The separation of the cartilages at the same time allowed the formation of more complex joint surfaces, as the articular cartilage no longer had any special growth functions (Vialleton, 1919).

Another factor has been the refinement of the processes of trabecular formation which has occurred in at least some lizards and in mammals. The cells of the typical growth zones have become arranged in more orderly columns and the marrow processes have been reduced to narrow elongated projections, each of which erodes a single cell column, and since all columns are eroded the cartilage between the processes is reduced to narrow trabeculae of matrix with no included hypertrophied cells. The change has been discussed in detail by Lubosch (1924, "intramedullare" and "endochondrale" ossification) and others. Other changes include the destruction in the bones of some mammals of most of the primary trabecular system and its replacement by a new system which is independent of the cartilaginous framework, and the development of cartilage canals.

Birds. In modern birds secondary centres are confined to the upper end of the tibio-tarsus (*Gallus*, Parsons, 1905; *Struthio*, *Rhea*, Fuchs, 1908).

In chondrodystrophies however, in which the advance of the zone of growth into the cartilage is retarded, a large crop of typical secondary centres may be developed at the ends of the long bones (Landower, 1927), so that the mechanism for the production of these centres is present though it is not normally used.

Further a rich system of cartilage canals derived from the perichondrium is found in the epiphyses of both the carinate *Gallus*, figured by Lubosch (1924) but described as marrow canals derived from the shaft, correctly indicated by Fell (1925), and in the ratite *Struthio*.

Now cartilage canals in mammals are usually, though not always, associated in late development with secondary bony centres whose tissues are derived from those of the canals (Hintzsche, 1928; Haines, 1933), and in the varanoid lizards, the only non-mammalian group besides the birds which possess large canals, I have found similar centres. So it is reasonable to assume that the birds also at one time had secondary centres in their epiphyses. Their loss was probably associated with the invasion of the bones by air-sacs, which in late development replace the marrow, and may project beyond the bony shaft (Blumstein-Judina, 1905). This has led to a re-approximation of the growth cartilage to the articular cartilage in late development.

Birds have never refined their trabecular arrangement as have the mammals, so that the scaffolding of calcified cartilage is thick and retains a large number of hypertrophied cells (Lubosch, 1924). They can therefore be used as convenient material for the study of the primitive mechanism of endochondral ossification, but the rest of their structure is highly specialized.

Urodeles. The structure of the bones of the urodele amphibians is better known than that of any other group except the mammals. Eggeling (1911), in the great monograph which has become the basis for all subsequent discussion of the evolution of epiphyses and of endochondral bone, has described a long series of forms including the whole range of living examples, and Klintz (1911) has published a valuable account of the microscopical anatomy of the bones in *Salamandra*.

Cryptobranchus (Fig. 11) is a good type which stands midway between the extreme neotenic forms in which the cartilage is never eroded even in the adult and the forms in which one large marrow cavity extends the whole length of the shaft. Further the endochondral bone is relatively well developed. The cartilage projects beyond the bony shaft as a hyaline mass with scattered undifferentiated cells (*z.u.c.*). In the growth zone, which lies opposite the end of the shaft, the cells are more closely packed and are slightly smaller (*z.g.*), but they are not flattened and they are not arranged in clumps or columns. In the succeeding zone the hypertrophied cells (*z.h.c.*) are clearly marked off by their size, and the matrix is calcified near the shaft (*p.c.c.*) and also in distinct layers which cross the axis of the shaft transversely (*t.l.c.c.*). These layers are probably due to some seasonal change in the composition of the matrix derived from the growth zone. No primary marrow cavity is developed in any bone, so that the hypertrophied cartilage can be traced from end to end of the shaft. The secondary cavities (*s.m.c.*) form a series of relatively small marrow spaces cut in the cartilage, never large enough to break its continuity. Endochondral bone (*e.b.*) is deposited as a more or less complete lining to some of the cavities, and often has no continuity with the shaft.

It is at once clear that the urodele structure differs profoundly from the primitive condition. The small amount of endochondral bone can have little functional importance, and its structure is clearly degenerate. Probably the whole structure is, as Miner (1925) has suggested for *Megalobatracus*, specialized for a secondarily aquatic life, though it is difficult to understand what advantage it can confer on the animal. Possibly a skeleton which is largely cartilaginous is less liable to fracture than a more bony one, so that when the limbs are not required to support the weight of the animal for long periods, a cartilaginous skeleton may have a biological advantage. In any case in tetrapods it is a specialized, not a primitive structure, though it had

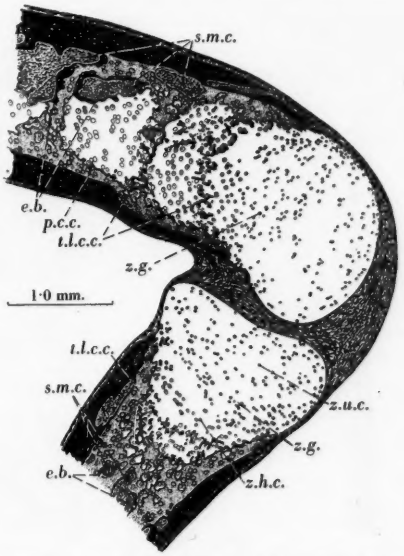

Fig. 11. Longitudinal section of the knee of *Cryptobranchus alleghaniense*.

already appeared in the carboniferous branchiosaurs, which may possibly include the ancestors of the living urodeles (Bulman & Whittard, 1926).

In the less specialized land-living amphibians the epiphysial structure approaches more nearly to the primitive condition. In Salamandra (Klintz, 1911) the cells of the zones of growth and hypertrophy show a poorly defined tendency to a longitudinal arrangement, the endochondral bone is scanty but is arranged in definite longitudinal trabeculae, and the interior of the shaft is occupied by a single large marrow cavity. But even this structure is very far from that of the primitive amphibians of the coal measures, with their plentiful endochondral bone arranged as a series of radiate trabeculae.

Anurans. A large number of anurans have been described by Fröbose (1927), who gives the earlier literature. They show many similarities to the

urodeles, some of which may be due to common descent, but the two lines were distinct by Jurassic times. The best known type is the common frog, *Rana temporaria* (Fig. 12). Here the epiphysial structure is similar to that in urodeles, but a rim of cartilage overlapping the shaft is enormously developed, giving the well-known "match-head" type of epiphysis. Calcification of the rim and of the cartilage beyond the shaft is continuous, so that the cartilage appears incapable of further interstitial expansion. In many places lines of apposition (*l.a.*) indicate the limit of calcification at an early stage in development. The growth cartilage (*z.f.c.*) contains scattered flattened cells and is sharply limited by the secondary epiphysial calcification distally, and by the

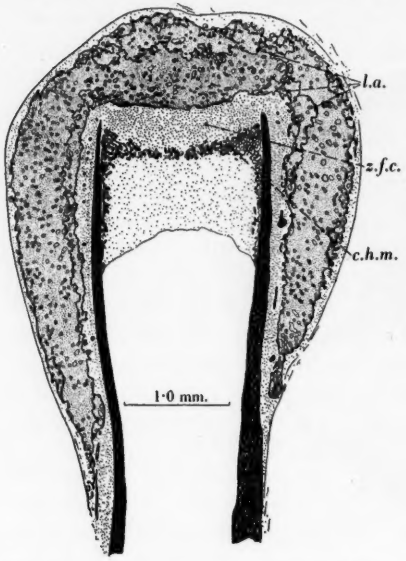

Fig. 12. Longitudinal section of the lower end of the femur of *Rana temporaria*.

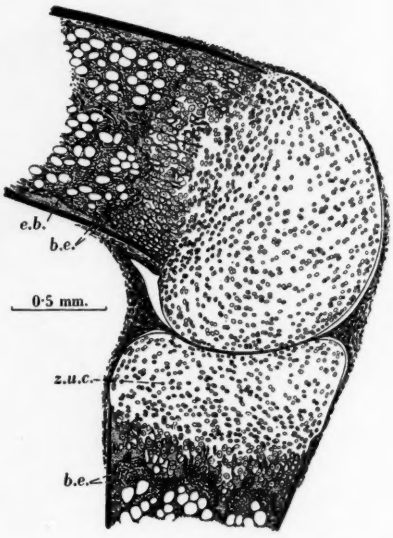

Fig. 13 Longitudinal section of the knee of a young *Xenopus laevis*.

calcified cartilage of the zone of hypertrophied cells (*c.h.m.*) proximally. There is no endochondral bone whatever, and the periosteal bone of the shaft is a simple smooth-walled cylinder.

The whole structure of *Rana* is clearly very highly specialized, but some anurans are less far removed from the ancestral condition. *Xenopus* is a secondarily aquatic animal and in some details, such as the persistence of lateral line organs in the adult, may be neotenic, but on the whole it is a good primitive anuran type. In the young animal (Fig. 13) the zone of undifferentiated cells (*z.u.c.*) forms a large mass of hyaline cartilage. The cells of the growth zone are scattered, but those of the zone of hypertrophy show some tendency to a vertical arrangement, and the zone is, as in primitive epiphyses,

fully calcified. The surface of erosion of the hypertrophied cartilage is not smooth as in *Rana*, but shows bays of erosion (*b.e.*) leaving remnants of calcified matrix projecting into the cartilage, on which endochondral bone (*e.b.*) may be laid down.

In an older example the epiphyses were more distinctly anuran in type, for the cartilage had grown back over the shaft as a typical rim, and it was heavily calcified. The zones of flattened and hypertrophied cells were similar to those in the younger animal, but the bays of erosion were better marked, and the endochondral bone well developed, resembling that in the half-grown tortoise. Thus this primitive anuran combines a well-developed system of endochondral bone with a typical form of epiphysis. Further the endochondral bone of *Xenopus*, and of other anurans in which it is present such as *Bufo* (Kastschenko, 1881) gives welcome confirmation to the thesis that a regular arrangement of the bone is a primitive feature in tetrapods. The living urodeles are more specialized in their endochondral bone than are the more primitive anurans; only the crocodiles and chelonians can be used as living examples of a relatively primitive form of epiphysis.

Secondary centres. Parsons (1905) divided secondary centres of calcification and ossification into those determined by pressure, by tension and by atavistic factors. He was undoubtedly right in supposing that mechanical stresses were the ultimate determinants of the position and shape of secondary centres. But the centres themselves now appear to be formed merely to strengthen the mass of undifferentiated cartilage between the articular region and the zone of growth, when these have become separated by a wide interval. On the other hand epiphysial structures as a whole are phylogenetically much older than secondary centres, and are related rather to the endochondral bone of the shaft and the general forces passing through it than to the local variations of pressure and tension.

CONCLUSIONS

1. The primitive form of tetrapod epiphysis is found with slight modifications in the living chelonians and crocodiles, but in no other living group.

2. The cartilage of the epiphysis in early tetrapods formed a rather thick hyaline mass over the end of the bone, with no secondary centres of calcification or ossification.

3. The growth cartilage was smoothly curved with its convexity towards the articular surface, and the cells were arranged in longitudinal clumps or columns.

4. The endochondral bone was well developed, forming a series of longitudinal primary trabeculae radiating towards the articular surface. In later growth they were modified, but never completely replaced by a secondary system of new trabeculae.

5. The modern amphibians are specialized in several directions, particularly

in the modification of the endochondral bone, and the details of their structure cannot be used to illustrate the primitive condition.

6. Other modern groups, including *Sphenodon*, lacertilians, birds and mammals, have progressed by such changes as the development of secondary centres of calcification or ossification, the vascularization of the cartilage by canal systems, the modification of the shape of the growth cartilage, the refinement of the early trabecular system and its replacement by a secondary system of new trabeculae, etc., none of which were found in the primitive epiphyses of early tetrapods.

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KEY TO LETTERING

Hyaline cartilage plain, calcified cartilage stippled, bone black.

a.b.e. apex of bay of erosion.
a.f.c. articular fibro-cartilage.
a.m.c. accessory marrow cavity.
a.p.p.b. accessory plate of perichondral bone.
art.s. articular surface.
a.unc. area of undercutting.
b.e. bay of erosion.
c.a.c.c. central area of calcified cartilage.
c.clm. cartilage column.
c.g.o. cell of globulus osseus.
c.h.m. calcified matrix of zone of hypertrophy
c.m. cartilage matrix.
c.z.h. calcified area of zone of hypertrophy.
d.w.b.e. divergent walls of bay of erosion.
e.b. endochondral bone.
e.b.e.b. endochondral bone formed at end of bay.
e.b.s.b. endochondral bone formed at side of bay.
eo.b. endosteal bone.
g.o. globulus osseus.
h.c.b. body of hypertrophied cell.
h.c.p. pit formed from capsule of hypertrophied cell.
i.h.c. isolated mass of hypertrophied cartilage.
l.a. line of apposition.
l.t. longitudinal trabeculae.
m.p. marrow process.
o.b. osteoblast.
ob.clm. cartilage column lying obliquely to plane of growth cartilage.
obl.t. trabecula meeting articular surface obliquely.
p.a.c.c. peripheral area of calcified cartilage.
p.b. periosteal bone.
p.c.c. peripheral calcified cartilage.
p.m.c. primary marrow cavity.
p.s. periosteal bone of shaft.
p.w.b.e. parallel walls of bay of erosion.
rt.ang.t. trabecula meeting articular surface at a right angle.
s.c.m. spicule of cartilage matrix.
s.e. surface of erosion.
s.g.o. stalk of globulus osseus.
s.m.c. secondary marrow cavity.
s.p.p.b. secondary process of periosteal bone.
tb.e.b. tube of endochondral bone.
t.l.c.c. transverse layer of calcified cartilage.
t.t. transverse trabecula.
uc.z.h. uncalcified region of zone of hypertrophy.
z.f.c. zone of flattened cells.
z.g. zone of growth.
z.h.c. zone of hypertrophied cells.
z.u.c. zone of undifferentiated cells.

A STUDY OF SPINAL CORD DEVELOPMENT IN SILVER-STAINED SHEEP EMBRYOS CORRELATED WITH EARLY SOMATIC MOVEMENTS

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A preceding paper (Barcroft *et al.* 1936) dealt with the course of development of early somatic movement in sheep embryos. The present article takes as its scope a histological study of growth changes in the rostral part of the spinal cord of these same embryos and attempts to interpret some of the early behaviour in the light of these changes.

Twenty-one sheep embryos ranging from 32 to 38 days of age were tested physiologically and their behaviour was observed at the physiological laboratory in Cambridge. Twelve were prepared for histological study after this had been done and forwarded to our laboratory in Chicago. Fifteen additional untested embryos ranging from 4 mm. to and including 20 mm. crown-rump length were obtained. All specimens were stained by the pyridine-silver method of Ranson, modified by reducing the time in some of the reagents (Davenport *et al.* 1934). Those studied in detail are listed in Table I.

Table I. *Material studied*

Embryo No.	Size mm., C.R. length	Age days	Embryo No.	Size mm., C.R. length	Age days
121	4	19±1	764	20*	?
113	7	?	136	23	32±1
117 A	8	22±1	144	23·5	33±1
760	12*	?	134	23·5	34±1
759	13*	?	146	24	34±1
761	14*	?	141	24·5	33±1
762	16·5*	?	147	25	34±1
763	16·5*	?			

* Approximate size determined after fixation. The other specimens were accurately measured before fixation.

OBSERVATIONS

Motor neurons. The precocity of the motor apparatus is even more clearly demonstrated in the sheep than it was in the cat (Windle, 1932 *a*, *b*). In the 4 mm. embryo neurofibrils are appearing in neuroblasts at the rostral end of the spinal and bulbar motor column. The cells are unipolar and bipolar and not until the 8 mm. stage do they start to become multipolar. In the 7 mm. embryo the cell bodies of the visceral motor nerves such as the vagus are found more medial and ventral than those of the somatic nerves such as the

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hypoglossal and the spinal ventral roots. By the time the sheep has reached a length of 16·5 mm. the visceral nuclei have shifted to their lateral stations. This process of migration has been described in cat embryos (Windle, 1933). There is no distinct subdivision of the spinal motor column in the smaller sheep embryos but at the 16·5 mm. stage the column has begun to divide into medial and lateral nuclei for axial and appendicular innervation respectively. Frontal and saggital sections demonstrate that motor nerve cells extend their processes in a plane at right angles to the long axis of the spinal cord. In the 16·5 mm. embryos the intermedio-lateral cell column of the thoracic region is just beginning to differentiate and in the 20 mm. embryo this cell group has definitely separated from the ventral horn. At the same time the cell bodies of the spinal accessory nerve have come to lie dorso-lateral to most of the ventral horn cells.

Internuncial neurons. Unipolar secondary neurons have differentiated in the lateral wall of the spinal cord of the 7 mm. embryo. Most are found in the alar plates but a small number are located more ventrally. The axons of all secondary neurons of the 7 mm. embryo course ventrad and give rise to a very small ventral spinal commissure and, on the opposite side, a longitudinal tract, the anlage of the ventral funiculus. This funiculus lies along the lateral side of the spinal cord superficial to the motor cell column. In the 8 mm. embryo the commissural fibres have increased in number and part of the ventral funiculus now lies ventral to the emerging motor root fibres (Pl. I, fig. 4). At 13 mm. the commissure and funiculus are well developed. In sheep embryos of 8 mm. and larger the commissure is found in all spinal cord segments. A study of frontal sections of the spinal cord indicates that the course of most of the commissural fibres after crossing the floor plate is rostrad.

Non-crossing associational neurons could not be observed in specimens smaller than 14 mm. long. The associational fibres arise from cell bodies in the alar plates and course laterad to enter into the formation of a lateral funiculus. In the 16·5 mm. embryos this direct system is well established. The axons of associational neurons course through the dorsal part of the brachial motor nucleus where they come in contact with dendrons of the motor cells. Prior to the 20 mm. stage local motor and internuncial elements form the predominant neuronal pattern of the spinal cord. As yet there is no indication of a significant downward growth of pathways from the brain.

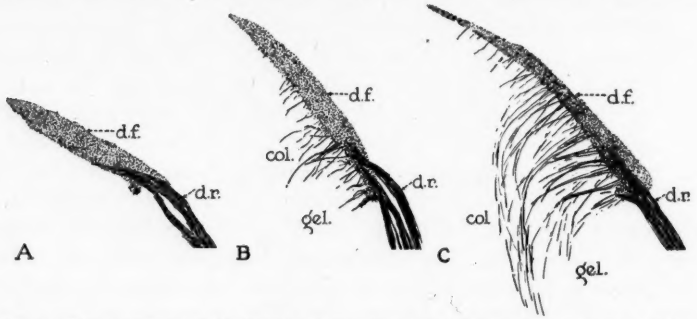
Sensory neurons. The development of the sensory lags behind that of the motor mechanism. Dorsal ganglion cells, roots and funiculus are lacking in the 4 mm. embryo. By 7 mm. a few bipolar neuroblasts are present in the ganglia (Pl. I, fig. 2). In the second cervical segment a few fibres from these bipolar cells enter the dorsal lateral part of the cord and course toward the central canal without building a longitudinal tract. In the 8 mm. embryo dorsal root fibres form the anlage of the dorsal funiculus (Pl. I, fig. 4). This funiculus is a prominent compact bundle of afferent fibres at 13 mm.

Dorsal root ganglion cells retain their bipolar character and the dorsal

funiculus appears as a sickle-shaped tract upon the dorso-lateral surface of the spinal cord in all the larger sheep embryos. In those of 20 mm. the substantia gelatinosa makes its first appearance as a cellular mass lacking fibrils and located just ventral to the dorsal funiculus where it displaces the associational fibres medially and ventrally.

Prior to the 20 mm. stage the tractus solitarius is not a separate bundle but appears to be located in the lateral and ventral part of the dorsal funiculus. It lies just medial and ventral to the dorsal funiculus at 20 mm. and is quite large in the 23 mm. embryo. The tractus solitarius receives fibres from the dorsal funiculus and the spinal accessory nerve in addition to those from the cranial visceral afferent nerves. The tractus solitarius also receives some associational fibres from the anlage of the recticular formation. It descends into the third cervical spinal cord segment.

It is clear that all intrinsic elements which go to make up simple spinal reflex arcs have been formed before the sheep reaches the 32nd day of gestation (23 mm.). Associational and motor neurons are closely related in position but sensory neurons, late in formation, are isolated from the other spinal structures in a compact tract upon the dorso-lateral surface of the cord.



Text-fig. 1. Detail camera lucida sketches of the dorsal funiculus and dorsal roots at the seventh cervical spinal cord segment of sheep embryos of (A) 20 mm., (B) 23 mm., and (C) 24 mm. C.R. length. Somatic movements were elicited in C but not in B; the sensory collaterals reach the association centre in C but not in B. Pyridine-silver stain; magnification ×72.

Table II. *Number of sensory collaterals in representative spinal cord segments*

Embryo No.	Age days	Size mm.	C 3	C 5	C 7	T 1	Total
136	32	23·0	3914	3567	4607	2761	14,849
144	33	23·5	6100	5982	6111	3569	21,762
134	34	23·5	3808	5544	6741	3859	19,952
146	34	24·0	7033	8710	9408	5009	30,160
141	33	24·5	2410	3604	5660	2428	14,102
147	34	25·0	7913	9289	9686	6182	33,070

In the 23 mm. embryo the first sensory collaterals have appeared along the medial border of the dorsal funiculus. At first they are short and most of them are located near the lateral end of the tract (Text-fig. 1 B and Pl. II,

fig. 6). The collaterals increase in number between 23 and 25 mm. Many swing mesad and ventrad around the substantia gelatinosa in specimens greater than 23 mm. long (Text-fig. 1 C and Pl. II, fig. 7), converge upon the associational nucleus and deploy among the dendrites and cell bodies of these internuncial neurons.

In order to obtain an index for comparing the degree of sensory development the collateral fibres of the dorsal funiculus were counted unilaterally in spinal cord segments C 3, C 5, C 7 and T 1 of a group of physiologically tested embryos. Results are shown in Table II. The greatest number of collaterals were encountered at C 7 in all embryos; the least, at T 1. It should be emphasized that all the embryos were darkly stained with the silver reduction and all are coloured to about the same degree with the exception of embryo No. 141 in which the stain was somewhat uneven. A binocular compound microscope with 2 mm. oil immersion objective was used. Both authors checked the results.

Peripheral nerves. The spinal nerves are well formed in the 13 mm. embryo and can be followed far toward the periphery. The growth tips of the brachial plexus fibres can be seen in the forelimb at 16·5 mm. Some terminate in groups among mesodermal cells of the premuscle masses and others ramify beneath the epithelium; no specialized endings of adult type exist. In the 20 mm. embryo some of the shoulder muscle are differentiating. Both sensory and motor nerves can be recognized. In the 23 mm. embryo the fibres which are running beneath the epithelium end in free endings and little bulbs upon the bases of the epithelial cells. Other nerve filaments enter muscles, run parallel to the myoblasts and terminate in little swellings and in clusters of bulbs resembling grape-like endings upon the myoblasts (Pl. I, fig. 5). In the 24 and 25 mm. embryos skeletal muscles are well differentiated and numerous motor nerve fibres appear in them; superficial cutaneous nerves ramify beneath the integumental epithelium in many places. Thus both motor and sensory nerve endings of a primitive kind are present at the time the embryos become motile.

DISCUSSION

The fundamental plan of spinal cord development is alike in all mammalian embryos studied (Windle, 1933, 1934; Windle & Baxter, 1936; Windle & Fitzgerald, 1937). Species differences are for the most part minor and seem to be of little significance. An exception is found in the substantia gelatinosa. This nucleus is similar in the sheep, cat and man but is more highly developed in the rat, occupying so much space in the dorso-lateral part of the spinal cord that the sensory collaterals must course a considerably greater distance to reach the associational centre than in the other animals. Correlated with this, local reflexes do not manifest themselves quite so early in the rat as in other mammals (Windle & Baxter, 1936).

Studies in silver-stained embryos of the several species of mammals clearly

demonstrate that motor neurons appear first, to be followed by internuncial and then by sensory neurons. That such a sequence holds in parts of the nervous system above the spinal cord was indicated in a previous article (Windle, 1935). It seems to be a general rule that neurofibrillar differentiation proceeds from efferent to afferent side within any specific reflex system. From the standpoint of functional studies it is clear that myogenic responses precede neurogenic.

The appearance of neurofibrils in a cell marks it a nerve cell. Neurofibrils form concomitant with the outgrowth of processes, the nerve fibres. No proof exists that the cell is better able to conduct immediately after fibrils develop than it was before but it is natural to assume that as the cell grows to resemble the neuron, as we know it in the adult organism, its property of conduction improves. Previous to the appearance of effector organs it will be difficult to demonstrate nervous function even in the cells with long processes filled with fibrils. For a short time after myoblasts and primitive motor nerve endings are developed it is still impossible to demonstrate nervous function as expressed by muscular activity even though muscular contraction can be induced by stimulating the muscles directly. Since many long motor, internuncial and sensory neurons can be demonstrated at this time by the silver technique, it is evident that something more than the development of neurofibrils in nerve cells and fibres must occur before reflex activity can take place. The present and other studies (Windle, 1934; Windle & Baxter, 1936) offer an explanation of the first embryonic movements in terms of anatomic structures.

The only fundamental structural change occurring in the spinal cord of sheep embryos between 20 and 25 mm. long involves the number and especially the length of the sensory collateral fibres. The 20 mm. embryo has no collaterals. The 23 mm. embryo has a large number but they are short and fail to reach the associational nucleus. In all embryos larger than 23 mm. long, collaterals increase in number and some reach the associational nucleus. The associational and brachial motor neurons bear intimate relationship throughout their development and are in structural contact. All the embryos larger than 23 mm. long which were studied histologically had responded to stimuli (Barcroft *et al.*, 1936). Faradic shocks applied to the back over the spinal cord were followed by forelimb movements. In those 24–25 mm. long mechanical stimulation (pressure upon the amnion) produced similar results. We believe that it was possible to obtain these responses because the specimens in question had reached a stage in development at which nerve impulses could flow from afferent to efferent neurons over newly formed collaterals in the spinal cord. That this correlation between structure and function is not simply coincidence is indicated by the fact that other species of animals (cat, rat and chick) have shown the same (Windle, 1934; Windle & Baxter, 1936; Windle & Orr, 1934).

The assumption of Barcroft *et al.* (1936) that two neurons and a synapse were involved in the embryonic forelimb reactions is conservative. The

histological observations allow us to suggest that three neurons and two synapses may have been involved. The impulse could have travelled over an afferent fibre of the dorsal funiculus and its collateral branches to a synapse with an associational neuron in the spinal cord, then to the brachial motor nucleus where a second synapse would have led to discharge of a motor neuron. The peripheral terminations of motor neurons on myoblasts are clearly formed.

Recent studies in cat embryos (Windle, 1937) have shown that localized stimulation of afferent endings in the fore paw produce responses like those seen in the sheep. These involve all the neural elements of simple unilateral, unisegmental, spinal reflex arcs from receptor ending to effector ending.

SUMMARY

A series of silver-stained sheep embryos demonstrate the precocity of development of the motor apparatus. As early as the 4 mm. stage neurofibrils are present in motor cells at the rostral end of the spinal cord and in the medulla oblongata. Internuncial neurons are first seen in the 7 mm. embryo. These form the commissural fibres and ventral funiculus of the opposite side of the spinal cord. The direct associational neurons were first observed in specimens 14 mm. long. Their axons enter into the formation of the lateral funiculus of the same side.

A few bipolar neuroblasts are present in the dorsal root ganglia of the 7 mm. specimen. At 8 mm. dorsal root fibres form the anlage of the dorsal funiculus which becomes a prominent tract at 13 mm. The first collaterals are given off to the grey matter by this tract in the 23 mm. sheep embryo. Earlier than the 23 mm. stage the associational and motor neurons are closely related in position and structural contact between them does exist, but the sensory nerve fibres are isolated from other intrinsic spinal cord structures in a compact tract upon the dorsal surface of the cord.

The peripheral nerves, both motor and sensory, and the myoblasts in certain muscle masses are well developed at the 23 mm. stage. Motor nerve fibres terminate in free endings and little bulbs upon the myoblasts. Free sensory endings are in contact with the epithelium in some places, notably the forelimb.

Prior to the 23·5 mm. stage physiological responses could be elicited only by stimulating muscle directly. Physiological activity which seemingly involved nerve conduction was elicited in the 23·5 mm. and all larger embryos. At this time some of the sensory collateral fibres have grown out from the dorsal funiculus and have reached the region of the internuncial neurons thus completing the first structural reflex arcs.

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KEY TO LETTERING

as.	associational neurons.	*gel.*	substantia gelatinosa.
as.n.	nucleus of associational neurons.	*l.f.*	lateral funiculus.
c.	ventral commissure.	*mot.*	motor cell column.
col.	collaterals of dorsal funiculus.	*mot.l.*	appendicular motor nucleus.
d.f.	dorsal funiculus.	*mot.m.*	axial motor nucleus.
d.r.	dorsal root.	*v.f.*	ventral funiculus.
d.r.g.	dorsal root ganglia.	*v.r.*	ventral root.
end.	endings of motor nerve fibres.	XI.	spinal accessory nerve.

EXPLANATION OF PLATES I AND II

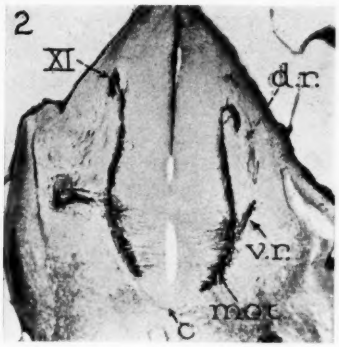
PLATE I

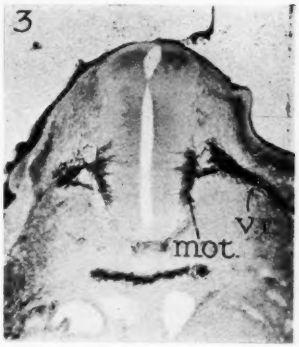
Unretouched photomicrographs of pyridine-silver stained sections of sheep embryos.

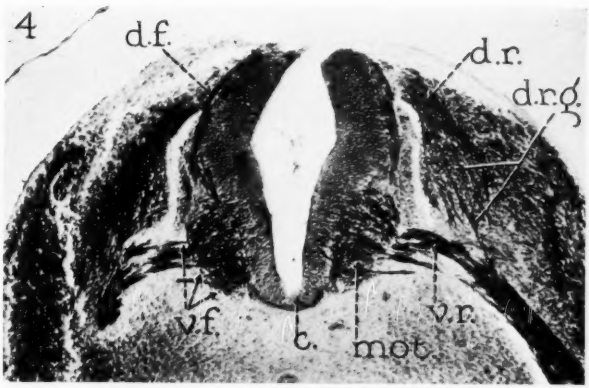
Fig. 2. The first cervical segment of embryo No. 113; 7 mm. long. The prominent darkly stained motor cell column and ventral motor root appears in the lateral part of the spinal cord. The spinal part of the accessory nerve is prominent in a dorsal position. The anterior commissure is faintly visible. The sensory dorsal roots and ganglion cells are beginning to form. Magnification: ×100.

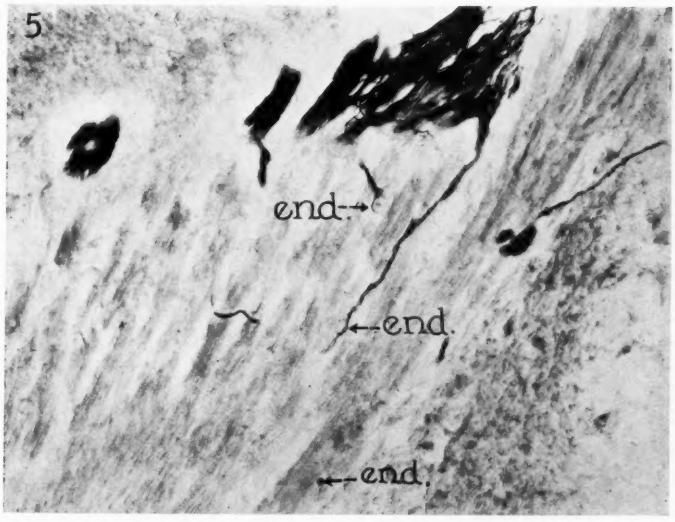
Fig. 3. The lumbar region of embryo No. 113; 7 mm. long. The motor columns and ventral roots are present but no sensory elements have neurofibrils. The anterior commissure is represented by two or three fibres which cannot be seen in the photograph. Magnification: ×100.

Fig. 4. The eighth cervical segment of embryo No. 117 A; 8 mm. long. Many sensory elements have appeared. The ventral commissure is larger and the ventral funiculus is seen on either side of emerging ventral roots. Magnification: ×100.









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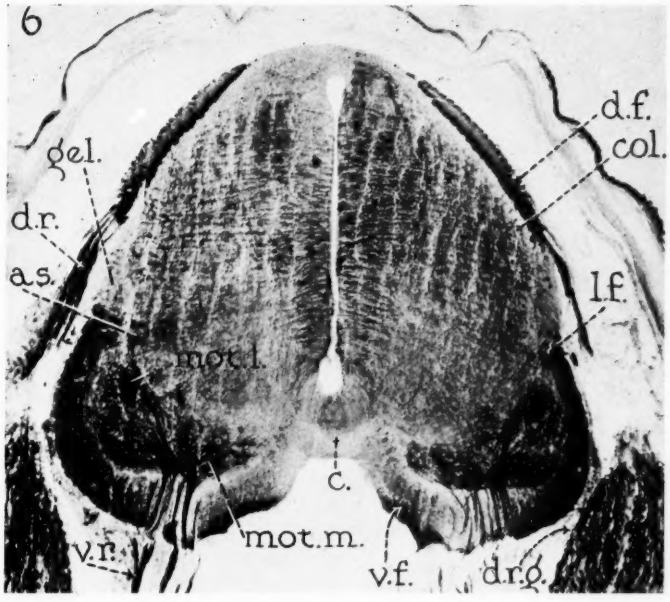
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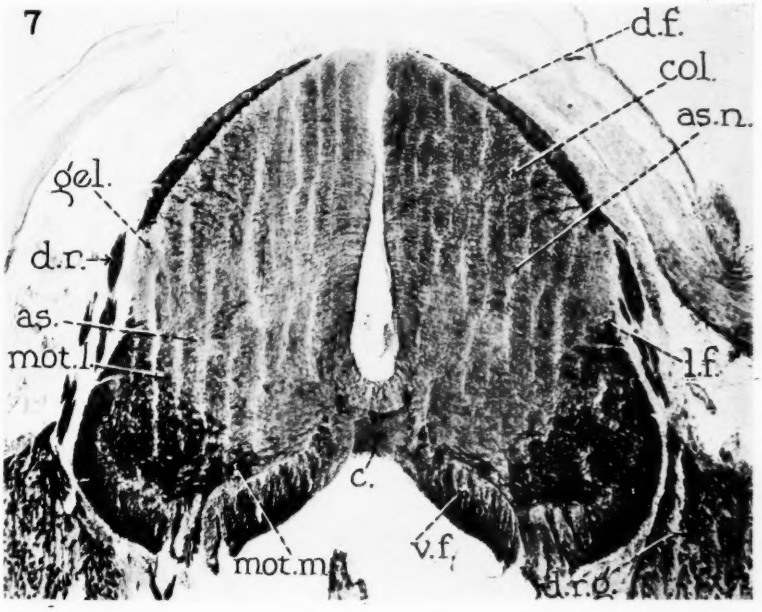
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Fig. 5. From a section through shoulder musculature of embryo No. 146; 24 mm. long. Myoblasts form a background against which motor nerve fibres and endings are seen. Magnification: ×320.

PLATE II

Unretouched photomicrographs of pyridine-silver stained sections of sheep embryos.

Fig. 6. The sixth cervical segment of embryo No. 136; 23 mm. long. The motor column has divided into medial and lateral nuclei. The associational fibres, not clearly illustrated, course into the prominent lateral funiculus. The dorsal funiculus is large and along its ventral edge a few short collaterals may be seen. The substantia gelatinosa is indistinct. Magnification: ×100.

Fig. 7. The eighth cervical segment of embryo No. 146; 24 mm. long; compare with Pl. I, fig. 4. Prominent in this specimen are the collateral branches of dorsal root fibres of the dorsal funiculus which course into the nucleus of the associational neurons. Fibres from this nucleus form part of the lateral funiculus adjacent to the appendicular (brachial) motor nucleus. Magnification: ×100.

OBSERVATIONS ON THE CERVIX UTERI AND THE URETHRA OF MONKEYS

By O. C. SANDYS and S. ZUCKERMAN

From the Department of Human Anatomy, Oxford

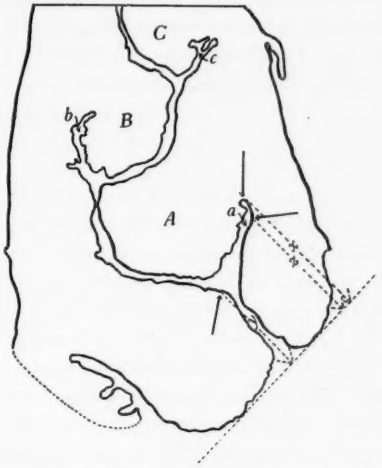
Clark and Corner (1935) have recently pointed out that the position of the line of junction of stratified vaginal and cylindrical cervical epithelium in the rhesus monkey (*Macaca mulatta*) is very variable. In one of the twelve specimens which they studied it was situated at the base of the so-called "cervical colliculus", and in another it was found at the lowermost point of the portio vaginalis. In general it appeared that the zone of transition rises with increasing age. Meyer (1910), however, who studied human material, states that the boundary between the two types of epithelium always lies in the cervical canal at first, and that as foetal life progresses the epithelium that differentiates into cylindrical cells "destroys the pavement epithelium as far as the external os uteri, and in about one-third of cases even further". The zone of transition is generally recognized to be unstable, and the term "physiological erosion" is applied to the not infrequent presence of patches of cylindrical cells on the vaginal surface of the portio vaginalis in women (Felix, 1912). Moreover, there is a tendency for the cylindrical cells of the human cervical canal to undergo metaplasia into squamous epithelium, a fact which is often related both to the frequency and to the type of new growths in that region, and which is of interest in view of the finding that similar metaplasia of the normal cervical epithelium can be induced in monkeys by the prolonged injection of oestrin (Overholser & Allen, 1933; Engle & Smith, 1935; Zuckerman, 1937 *a*).

The following study was accordingly undertaken to inquire (*a*) whether or not any orderly process can be recognized in the movement of the zone of transition of the two types of epithelium during the growth of the rhesus monkey, and (*b*) whether or not "cervical erosion" occurs normally in this species of primate.

MATERIAL AND METHOD

The material available for study comprised median sagittal sections (stained with haematoxylin and eosin) of the cervix uteri of forty-eight rhesus monkeys. Fourteen were young animals that had no history of uterine bleeding. Five of these fourteen had been injected previous to autopsy with oestrone (either alone or in combination with other gonadal hormones). Seven of the thirty-four animals in which at least one phase of uterine bleeding had been observed in the laboratory had also been similarly injected for varying periods.

Each section was examined microscopically under low and high power, and the points of junction of cylindrical cervical and squamous vaginal epithelium were marked in ink. Magnified projections of the cervical canal were then traced on white paper. The lowermost points of the ventral and dorsal cervical lips were connected by a straight line, and the vertical distances from this line to the points of epithelial transition on the ventral and dorsal cervical walls were measured (y and z, Text-fig. 1). The vertical distance



Text-fig. 1. Projection of sagittal section of cervix of rhesus monkey (OM 96). *a*, cul-de-sac caudal to main colliculus *A*; *b*, cul-de-sac below ventral colliculus *B*; *c*, cul-de-sac below inconstant dorsal colliculus *C*; *x*, vertical distance from tangential line connecting cervical lips to fundus of cul-de-sac "*a*"; *y* and *z*, heights of dorsal and ventral zones of epithelial transition respectively.

between this tangential line and the base of the cul-de-sac on the under surface of the main cervical "colliculus" was also measured (x, Text-fig. 1). The ratio of the mean of the first two dimensions to the third dimension was estimated and expressed as a percentage, which gives an approximate index of the height of the zone of transition. Other indices were tried, but were abandoned as being less suitable owing to difficulties created by differential growth in the cervix and uterus during the process of maturation of the rhesus monkey.

GENERAL OBSERVATIONS

The main cervical "colliculus" in the rhesus monkey (*A*, Text-fig. 1) projects as a plug from the ventral cervical wall into the cervical canal, which is not straight as in man. A cul-de-sac of the cervical canal, which is directed cranially in the dorsal wall of the cervix, has its mouth opposite the point of junction of the cranial and dorsal surfaces of the main colliculus (*b*, Text-fig. 1). This cul-de-sac, which was present in all of our forty-eight specimens, cuts off a

part of the dorsal cervical wall that may be regarded as forming a second "colliculus" (*B*, Text-fig. 1). Another cul-de-sac (*c*, Text-fig. 1), which is not referred to by Clark & Corner, is present in twenty of our specimens. It has its mouth at the base of the cranial surface of the main colliculus and is directed cranially in the ventral wall. This cul-de-sac demarcates a third "colliculus" (*C*, Text-fig. 1).

The subsidiary "colliculi" *B* and *C* to some extent influence the direction of the cervical canal (see Text-fig. 1). The main curvature of the canal is, however, determined by colliculus *A*, round which it bends, with a dorsal convexity, before it opens at the external os. The part of the canal below the main colliculus is not always straight. In about 20 % of cases it is curved, with a ventral convexity. The length of this distal part of the cervical canal was taken as the distance between the most dependent part of the main colliculus in the cervical canal and the line connecting the tips of the dorsal and the ventral lips of the os. Values for this dimension in the groups of animals that had menstruated are both greater and smaller than in the group of immature animals that had not reached reproductive maturity. It is of interest that the distal part of the cervical canal is shortest in two of the largest of the mature animals (OM 165, OM 128).

The dorsal lip of the external os is almost always thicker than the ventral, and as a general rule the dorsal fornix is deeper. The ventral fornix is undeveloped in some of the younger specimens. It may be noted, too, that the line connecting the tips of the cervical lips is in general more oblique in relation to the axis of the vagina in the younger than in the older specimens, and that the ventral and dorsal cervical lips vary to the same extent in respect to the depth of their projection into the vagina. In so far as it is possible to judge from sections, it would therefore seem that the uterus of non-pregnant rhesus monkeys is as often anteverted as retroverted.

A recto-vaginal fistula was found in one specimen, OM 128 (Plate I, fig. 1). The animal was apparently parous, and although vaginal washes had been examined every alternate day during the thirteen months it had lived in the laboratory, the existence of this condition was not suspected. The rectum also opened to the exterior by a normal anal canal and anus. The fistula led into the posterior fornix. The epithelium of the fistula, and of the rectum over the cervix uteri, is stratified squamous, and is therefore presumably related to the ectodermal proctodaeum (the epithelium of the lower rectum in the rhesus monkey is normally glandular). Moreover, the epithelia of the "rectum", the recto-vaginal fistula and the vagina are continuous and are identical in appearance. There is as much shedding of superficial cornified epithelium in the rectum as in the vagina.

Similar anomalies in human anatomy are extremely rare, for a rectovaginal fistula is usually associated with non-union of the hind-gut and the proctodaeum. Apparently there is no clear embryological explanation for the coexistence of a recto-vaginal fistula and an otherwise normal rectum and anal canal.

MICROSCOPIC OBSERVATIONS

(*a*) *Zone of transition of vaginal and cervical epithelium*

Owing to the gradual divergence of the cervical lips in the distal part of the cervical canal (see Text-fig. 1 and Plate I, fig. 1), it is impossible to mark the caudal limit of the external os with any accuracy, and for that reason the zone of epithelial transition was measured, as described above, in relation to a tangential line connecting the caudal limits of the ventral and dorsal lips The points of transition in all but four of our specimens are within the area enclosed by the cervical canal and this tangential line; in two they are on the tips of the cervical lips as viewed in sagittal section; and in two they are external to these points.

The zone of transition in both immature and mature animals lies as a rule within the narrow part of the distal cervical canal. In ten of the forty-eight animals the ventral transition occurs within the cul-de-sac that demarcates the main colliculus caudally. Most of these ten exceptions are mature animals in whom the distal part of the cervical canal is relatively short. In thirty-six of the forty-eight specimens the line of transition is higher on the ventral than on the dorsal cervical wall; in four the levels of transition are the same on both sides; and in eight that on the dorsal wall is higher.

The forty-eight specimens we studied do not suggest that there is any orderly movement of the zone of epithelial transition with age—whatever be the index by which the line of transition is denoted. Moreover, there appears to be no correlation between the transition-index and the distance between the most dependent part of the main colliculus and the external os. It is significant that a fully mature and an immature monkey were the two animals in which the zone of transition was most distantly situated on the vaginal surface of the cervix.

(*b*) *The nature of the zone of transition*

As noted above, the term "physiological erosion" of the cervix strictly speaking refers to the presence of cylindrical epithelium on the vaginal surface of the cervix uteri. On this definition erosion existed in several of our normal specimens, in particular in OM 106, one of the oldest. In only thirty of the forty-eight specimens was the transition from stratified vaginal epithelium to cylindrical cervical epithelium abrupt. In the remaining eighteen cervices aberrant islets of either squamous or cylindrical epithelium were present on either side of the main line of transition. Only one of these eighteen animals had not experienced uterine bleeding.

The usual type of epithelial mixture shows patches of squamous stratified cells in the fundi of the cervical glands (Plate I, fig. 2). These plugs of cells are essentially the same as those which characterize the epithelial metaplasia that is induced by prolonged oestrogenic stimulation, and it is significant that they occur in animals which have not been injected with sex hormones. It is also

important that patches of squamous cells may be found relatively far from the main line of transition.

The caudal surface of the main colliculus is covered by stratified epithelium in four animals (Plate I, fig. 3), of which two, OM 79, MM 155, were adolescent. The other two were fully mature (128, 139), and in them the colliculus almost projects out of the external os.

(*c*) *Lymphocytic nodules in the urethra of monkeys*

Collections of lymphocytes are frequently seen deep to the urethral epithelium in male rhesus monkeys (*Macaca mulatta*) that have been injected with oestrone (van Wagenen, 1935; Zuckerman, 1937 *b*). They are more numerous in the upper urethra, and they increase in extent the longer injections are given. Examination of different levels of the urethra of twenty normal animals belonging to this species showed that similar but smaller lymphocytic "nodules" are frequently present in animals that have not been injected with oestrone, and that they are commonest in the prostatic region. They are present both in sexually mature and in sexually immature monkeys. Their frequency does not appear to increase after the injection of male hormone, although the small number of specimens in our series which were so treated (ten) does not permit a definite conclusion on this point.

There is no reference in standard text-books to the presence of similar nodules in man, and the only mention in the literature of their occurrence in normal men that we have succeeded in finding is a paper by Busch (1905), who observed them in six of twenty specimens, and mostly in the fossa navicularis. Urethral lymphocytic nodules are present in subhuman species of primate other than the rhesus monkey. Thus they were found under the epithelium of the prostatic urethra in a normal green monkey (*Cercopithecus aethiops sabaeus*), a common macaque (*Macaca irus*) and a Hamadryas baboon (*Papio hamadryas*). Similar nodules were seen in a drill (*Mandrillus leucophaeus*) and a capuchin (*Cebus fatuellis*) that had been injected with oestrone. None was present in the following animals: a normal Mozambique vervet monkey (*Cercopithecus aethiops pygerythrus*), a normal and an oestrone-injected Mona monkey (*Cercopithecus mona*), a normal and an injected Hanuman langur (*Presbytis entellus*), a normal and an oestrone-injected Guinea baboon (*Papio papio*), and a marmoset (*Hapale jacchus*). It may be noted that in general the prostrate in these various species is the same as that of the rhesus monkey (*Macaca mulatta*). In the Mona and Mozambique cercopitheques, however, some compound tubular glands, which in staining reactions appear different from the main prostatic glandular tissue, are situated ventral to the prostatic urethra. Similar systems of glands are present in the capuchin and marmoset, and in the former the crista urethralis is extensively invaded by columns of presumably urethral epithelium. The urethra in some species, for example the drill, is much more widely dilated than in others.

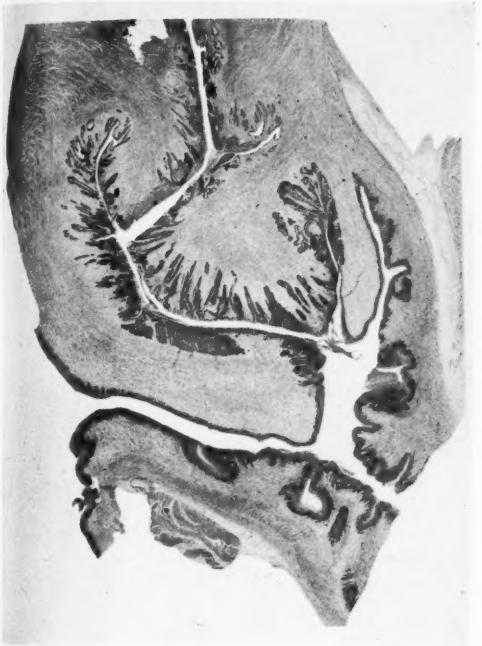


Fig. 1.

Fig. 2.

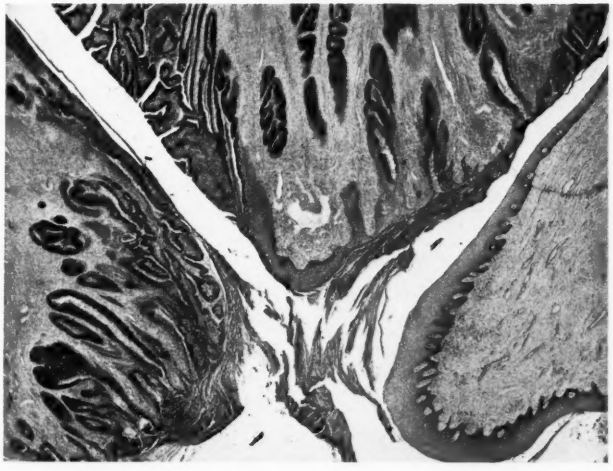


Fig. 3.

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Submucous lymphocytic nodules were not seen in the urethra of female monkeys. They are not uncommon in the dermis of the vestibule. None was seen in association with Skene's (urethral) tubules, which, though uncommon in the rhesus monkey, can usually be found in monkeys of the genus *Cercopithecus*.

SUMMARY

Two small and one large colliculus project into the cervical canal of the rhesus monkey. The large colliculus from the ventral wall and the single small colliculus from the dorsal wall are constant. The third and most cranial colliculus is cut off from the ventral wall. It is very small and is present in about 40 % of cases. The line of transition of cylindrical cervical and vaginal squamous epithelium varies in position independently of age. It may be situated anywhere between the vaginal surface of the cervix uteri and the apex of the cul-de-sac below the main colliculus. Clumps of stratified cells may be found in normal monkeys in the deepest parts of cervical glands above the main line of epithelial transition, and in four of forty-eight specimens the distal surface of the main colliculus was covered with stratified squamous and not with the normal single layer of cylindrical epithelium.

The occurrence of lymphocytic nodules under the urethral epithelium of various species of monkey is described.

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EXPLANATION OF PLATE I

Fig. 1. Cervix of OM 128, showing recto-vaginal fistula. × 3.
Fig. 2. Cervical glands of normal rhesus monkey (OM 18) showing stratified cells in the fundus of the glands. × 28.
Fig. 3. Stratified squamous epithelium covers the caudal face of the main colliculus of OM 128. × 18.

SOME OBSERVATIONS ON THE FOETAL VESSELS OF THE HUMAN PLACENTA WITH AN ACCOUNT OF THE CORROSION TECHNIQUE

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The literature dealing with the angio-architectonics of the foetal vessels in the placenta is surprisingly small. Indeed, textbooks of anatomy, embryology, and obstetrics make the briefest references to the subject, yet it is impossible to understand the physiology of the circulation in this organ without having first of all a clear idea of the anatomical relationship of its blood vessels.

The human placenta commences to function during the third month, and, according to most investigators, shows well-marked areas of degeneration at the end of pregnancy. It belongs to the haemo-chorial type, which according to Grosser represents the highest degree of development, and it is a unique example of the intimate and efficient functional union of the tissues of two separate individuals.

We know that the development of the vascular system of other organs is regulated by functional influences and hereditary factors, while in the case of the placenta, the site of implantation in the uterus and the characteristics of the maternal circulation also play an important role.

It is obvious that a clear conception of the foetal vessels in the placenta cannot be obtained by an ordinary dissection, and we believe that misconceptions are bound to arise from X-ray photographs taken after the vessels have been injected with an opaque material, for this method does not show the anastomoses of the blood vessels or their stereoscopic arrangement. On the other hand, the corrosion technique provides specimens which may be seen and handled, and, accordingly, is obviously superior.

TECHNIQUE

It is essential that the material should be obtained fresh, not more than 24 hours after death, and it should be unfixed.

If blood vessels are to be injected, they should be first washed through with acidulated water, in an endeavour to haemolyse any blood clot which might block the lumen of a vessel.

After this, it is an advantage in certain organs, e.g. the placenta, to distend the vessels by pumping in air, but great care must be exercised to prevent their rupture. The blood vessels of more delicate organs, such as the kidney, will certainly not withstand such severe treatment, and here the use of air is contra-indicated.

The next step is to choose a suitable injection material, and for this purpose we have used celluloid dissolved in acetone. We used the celluloid from old X-ray and "ciné" films, from which the gelatine layer had been removed with hot water. We find celluloid just as good as its purer form celloidin, and it has the further advantages of dissolving more readily and of being considerably cheaper. The strength of the solution depends on the material to be injected, e.g. for the bronchial tree, the solution should be thick, say about 20 %. If a weaker solution is used, not only the fine bronchioles but also the alveoli are filled and so the whole structure of the bronchial tree becomes obscured. On the other hand, for demonstrating the fine branches of an organ's vascular tree, we injected pure acetone first, followed by an injection of weak celluloid, the strength of which was gradually increased to 20 %. It is important to bear in mind that the acetone readily diffuses through the tissue spaces, whereupon the celluloid, now freed from acetone, solidifies. Thus if, at first, too thick a solution is used, the finer branches of the blood vessels will not be injected at all, because the celluloid will have solidified before reaching them.

For small organs the injection was done with a glass Record syringe, but for large organs a large metal syringe with a screw drive piston was used. Special nose pieces were made for the metal syringe with a stopcock at the end to maintain pressure after removing the syringe. Recently, American workers have driven in the injection mass by means of compressed air regulated by a mercury manometer, and with characteristic American appreciation of labour-saving devices, they describe a mechanical shaker for dissolving the celluloid in the acetone, but we are not sure that these more elaborate methods are justified by the results.

Undoubtedly the great secret of success is patience, because the injections require a considerable time—6–12 hours for small organs, and 1–2 days for large organs, a fairly even pressure being essential throughout.

When more than one system is to be injected in any organ, the injection mass must be coloured, and the choice of the colour medium is one of the most difficult problems in the technique. The selection of the stain or pigment depends upon the macerating medium employed—the two questions are inseparable. There are two groups of colour media: (1) *stains* soluble in acetone, e.g. Sudan III, Scarlet Red, the colours mentioned by Narat and others, and Berlin Blue, Prussian Blue, Asphalt, Alkanin and Crystal Violet, mentioned by Morison and others; (2) *pigments* insoluble in acetone, e.g. Cobalt Blue, Chrome Yellow, Cinnabar, Victoria Blue, as used by Morison.

Using the acetone-soluble dyes, we found that after injecting the veins and arteries in the same organ with different colours, a certain diffusion always took place which confused the result. Therefore, to inject more than one system in a specimen, we preferred to use acetone-insoluble pigments in the form of artists' oil colours, in which the pigment is so finely divided that no precipitation occurs even when the injection solution stands for several weeks.

After completing the injection it is necessary to immerse the specimen for

about 24 hours in water, as this facilitates the complete removal of the acetone and so enables the celluloid to harden.

The final stage is the maceration, and four possible methods are available.

(1) Commercial HCl either concentrated or diluted with one-sixth water is most generally used. By this method maceration is complete in from 1 to 6 days. This is certainly the quickest method, but unfortunately it bleaches most of the stains. We find, however, that Scarlet Red and Sudan III are acid-fast even after prolonged exposure in the concentrated acid. Narat and others claim to have obtained acid-fast dyes (Luxol Fast Blue AR, Orange E, Scarlet RR, Yellow, Red B, products of Du Pont de Nemours and Co.) but unfortunately we were unable to obtain these dyes.

(2) By ordinary macerating methods—this will not affect the dyes but it is very slow and not always completely successful.

(3) By pepsin and HCl digestion, but it is slow and expensive owing to the high price of pepsin.

(4) By 1 % KOH in an incubator at 56° C. This is rather slower than the HCl but it has the advantage that most colours are unaffected.

When maceration is complete, the specimens are soaked in hot water for a short time. The adherent tissues are then washed away with a strong current of water. After this, the specimen is further washed in running tap water for 24 hours to remove all traces of HCl or KOH.

Finally, the specimens may be preserved in a formaldehyde-glycerin or glycerin-spirit mixture as suggested by various authors—on the other hand, we prefer dry mounting.

We also found it an advantage to varnish our dry mounted specimens with a solution of resin in a petrol-chloroform mixture (petrol 5 parts, chloroform 1 part). This preserves the specimen, and enhances its appearance by brightening the colours of the pigments used for the injection.

OBSERVATIONS

Our chief attention was directed towards the umbilical arteries, and these vessels were injected in fifty placentae. The relations of the umbilical vein were studied in ten cases and either the vein alone or both vein and arteries were injected in these specimens.

We considered three main principles in regard to the arterial system, viz.:

(1) the pattern of the vessels,

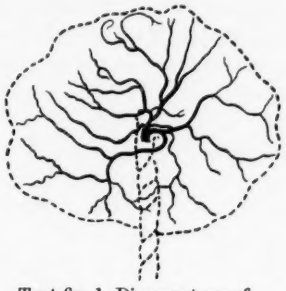
(2) the area of placenta supplied by each artery,

(3) any connexion between the two vessels and their branches.

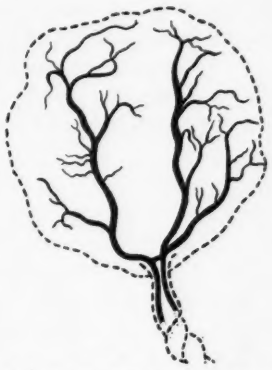
(1) The umbilical arteries enter the placenta either marginally or centrally. Here the arteries break up into several branches which run towards the margin of that organ, giving off further branches along their course. All the large arterial branches are in the same horizontal plane, and they lie immediately below the amnion.

According to the pattern of the arteries, the placentae may be divided into

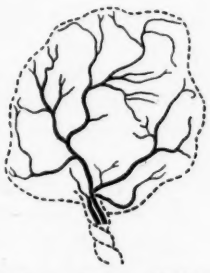
two main types: (1) a *disperse* type (Text-fig. 1), in which the two arteries divide dichotomously, and rapidly diminish in calibre, resembling in their spider-like arrangement the spokes of a wheel; (2) the *magistral* type (Text-fig. 2), in which the two arteries extend almost as far as the margin of the placenta before their calibre diminishes. Some of the placentae were definitely of a border-line type, and in these cases their classification needed more careful consideration.



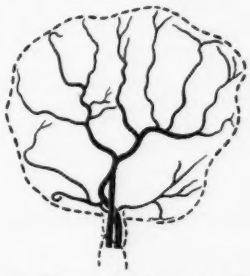
Text-fig. 1. Disperse type of placenta.



Text-fig. 2. Magistral type of placenta.



Text-fig. 3. Asymmetrical type of placenta.



Text-fig. 4. One umbilical artery is supplying the periphery of the placenta and the other the centre.

(2) The areas supplied by each of the arteries showed great variation. In twenty-seven cases equal areas of the placenta were supplied by each umbilical artery, that is, they were symmetrical. In twenty-three cases the distribution was asymmetrical, e.g. one-third would be supplied by one artery and two-thirds by the other (Text-fig. 3). In some cases, one artery supplied the peripheral part of the placenta while the other supplied the central part (Text-fig. 4).

It is important to emphasize the fact that whatever the relation of the areas supplied by the two arteries, these vessels were always of equal calibre in the cord.

(3) We found that there is a constant communication between the two arteries either immediately before or immediately after their entrance into the placenta. In forty-three of the specimens examined we found a well-marked transverse communicating branch. Sometimes this was as wide as the main branches, and at other times it was considerably narrower. This branch usually arose before the arteries branched, but it happened sometimes that one or two small branches came from one or other of the umbilical arteries before they were united by the transverse branch (Text-fig. 5). In some specimens branches arose from the transverse branch and at times these were quite large (Text-fig. 6). In seven of our specimens the actual transverse branch was missing, but in these cases the umbilical arteries were fused at the entrance to the placenta and then separated later (Text-fig. 7).

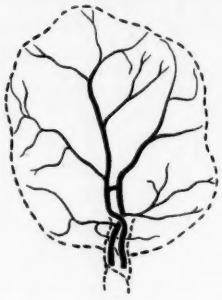


Fig. 5.

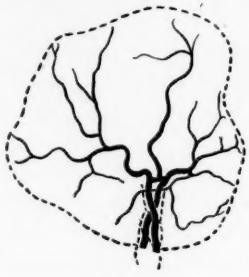


Fig. 6.

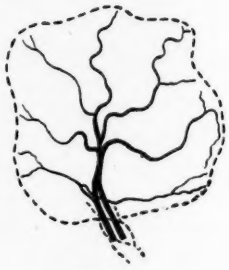


Fig. 7.

Text-fig. 5. Small vessels arising from each umbilical artery before they are united by the transverse communicating branch.

Text-fig. 6. A branch arising from the transverse communication.

Text-fig. 7. Fused umbilical arteries.

In our specimens we could see no sign of a peripheral anastomosis and we do not believe that the two arteries communicate except by the transverse branch.

The umbilical vein accompanies the arteries, but it is wider in calibre and more deeply situated (Pl. I, figs. 1 and 2).

The capillaries lie in the cotyledons and communicate so freely with both the arteries and veins that they can be readily injected through either system. In a well-injected specimen (Pl. I, fig. 3), the capillary network of an individual cotyledon resembles a ball of cotton-wool.

DISCUSSION

In discussing the literature dealing with the subject, it is interesting to note that Spanner (1935), using corrosion specimens, first pointed out that all the main arterial branches of the placenta lie in a horizontal plane, while, in most of the other organs of the body, the arterial branches are arranged in three dimensions. As already indicated, we were able to confirm this statement.

Fraser (1923) described the arteries of the human placenta as being similar in their arrangement to the spokes of a wheel. He did not allude to the fact later observed independently by two other authors, Burg (1929) and Schordania (1929), that all the placentae can be divided into two main groups, according to the pattern followed in their branchings. While these two authors give practically the same descriptions, the terms "disperse" and "magistral" were introduced by Schordania. It is clear that if Fraser's description is accepted, all the placentae examined by him must have belonged to the disperse type. These three authors, Fraser, Burg and Schordania, used X-ray photography in their researches, and our results on the corrosion specimens fully justify the descriptions given by the two latter workers.

Spanner was mostly concerned with the circulation in the intervillous spaces, and in his explanation of the foetal part of the circulation of the placenta, he attributes a special role to the finer branches of the umbilical veins, which, according to his microscopic investigations, contain a special "sluice-gate" and pressure-regulating mechanism. As we were not concerned with the microscopical characteristics of the foetal vessels of the placenta, we cannot deny the truth of his statements, but certainly on our corrosion specimens we were not able to observe the beaded appearance of the finer branches of the umbilical veins mentioned by Spanner as being seen on his venous corrosion specimens.

Schordania advanced the theory that placentae belonging to the magistral type are usually associated with a better-developed foetus, with greater weight, length and thoracic measurement, than those of the disperse type. He, as well as Fraser and Burg, agreed that the vascularity of the cotyledons shows regressive changes (white infarct) at the end of pregnancy. They also agreed that during pathological conditions of the mother the decrease of vascularity can develop at a much earlier stage, and may account for the death and abortion of the foetus. None of these authors advanced any theory regarding the physiology of the foetal circulation of the placenta.

Fraser, Hyrtl (1870) and Holl (1881) mentioned numerous anastomoses in the peripheral branches of the umbilical arteries, but we were not able to confirm this in our specimens, and in our opinion all the arterial branches distal to the transverse communicating branch are end arteries. This anatomical fact explains the occurrence of white infarcts.

Certainly our most interesting finding is the transverse communicating branch between the two umbilical arteries. With the exception of Burg, we are not aware that any previous writer has mentioned the presence of this branch, and Burg does not attribute any importance to it, while we believe it plays a most important role in regulating the circulation of this organ. The function of the circle of Willis is usually considered to equalize the pressure and establish an equal blood supply to all parts of the brain (see Cunningham and Buchanan). May we not apply the same functional role to this transverse communication? To support this view, when we remember that even the normal,

and certainly the pregnant uterus, carries out rhythmic contractions, it is obvious that during the contraction wave the blood pressure in the corresponding part of the intervillous space and cotyledons of the placenta is increased, and the presence of a pressure-equalizing or "buffer" system is well justified. This arrangement may also be compared with the arterial arcades of the intestines, where the continuous peristaltic movement is comparable to the contractions of the uterus.

The presence of the transverse communication between the two umbilical arteries also explains why these vessels are always equal in calibre, irrespective of the size of territory supplied by them, and therefore we must accept the conclusion that this transverse branch plays an active role in the distribution of the foetal blood in the placenta.

SUMMARY

The foetal vessels of fifty human placentae were studied on corrosion specimens. A detailed account of the corrosion technique is given.

1. According to the pattern made by the arteries, placentae can be divided into two groups: (i) a disperse type in which the blood vessels divide dichotomously, and (ii) a magistral type in which the two main arteries extend as far as the margin of the placenta and give off two branches of small size only.

2. Whatever is the relation of the areas supplied by the two arteries (symmetrical or asymmetrical distribution) these vessels are always of equal calibre in the cord.

3. There is a constant communication between the two arteries as they enter the placenta. Forty-three specimens showed a well-marked transverse communicating branch, while in seven specimens the two vessels were fused.

4. Because of the absence of peripheral anastomoses, it is believed that all the vessels to the placenta are end-arteries.

5. The authors believe that the transverse communicating branch establishes an equal distribution of blood and regulates the pressure in the placenta, counteracting as a buffer system the effects of uterine contractions,

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EXPLANATION OF PLATE I

Fig. 1. Distribution of umbilical vein in the placenta.
Fig. 2. Arteries and vein of placenta injected with different colours to show their respective relations.
Fig. 3. Injection of capillaries in the placenta.

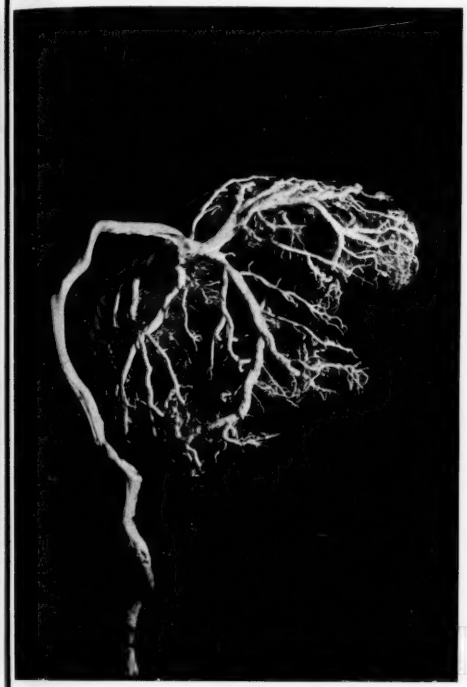

Fig. 1.

Fig. 2.

Fig. 3.

UNIV. OF MICH.

THE DEVELOPMENT OF THE SUPRA-UMBILICAL PORTION OF THE ANTERIOR ABDOMINAL WALL

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This work is a continuation of the study of the development of the ventral body wall, the first part of which, "The development of the infra-umbilical portion of the abdominal wall, with remarks on the aetiology of ectopia vesicae", was published in the *Journal of Anatomy* of January 1937.

The supra-umbilical portion of the anterior abdominal wall develops *pari passu* with the ventral growth of the liver, although, as is apparent from cases of eventration, a normally developing liver is not the controlling factor in the completion of this part of the parietes.

Up to and including a 4·5 mm. embryo the umbilical opening, invested by the amnial reflexions, has, as its superior limit, the mesodermal mass at the caudal end of the pericardium which more dorsally separates the pericardial from the abdominal coelom. In an embryo measuring 7·5 mm. and more emphatically in one of 12·5 mm. there is a definite interval between the pericardium and the upper border of the umbilical opening, a supra-umbilical wall. The mesodermal basis of this portion of the ventral body wall would seem to be a derivative of the general mass of mesoderm forming a partition between heart and liver, and therefore an endeavour to assess its developmental significance includes an account of the origin of the formations known collectively as the septum transversum.

The Glasgow collection of embryos has again been utilized. The appropriate history of each will be found in the first part of this work. A recent addition to the collection, an embryo 2·6 mm. in length, is incorporated in the series.

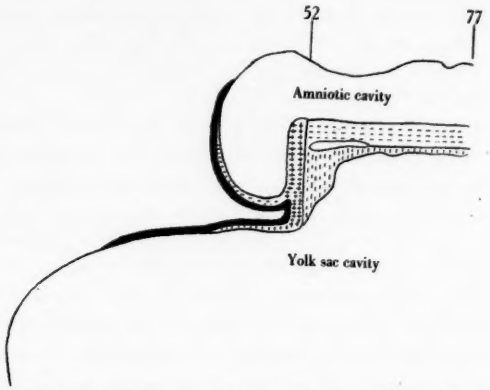
EMBRYO McINTYRE I

In embryo McIntyre I, the youngest embryo of the series (see Bryce, 1924), the head process is continued into the prochordal plate (Text-fig. 1), and cranial to this the ectoderm and endoderm are in contact, a buccopharyngeal membrane lateral to which are the mesodermal sheets (Pl. I, fig. 1).

In front of the head fold (Section 50, Pl. I, fig. 2) the ectoderm and endoderm are separated by mesoderm forming a continuous sheet across the middle line and exhibiting cleavage with the formation of a number of small spaces, precursors of the pericardial coelom. This mesoderm at the anterior end of the disc and in the region of the cranial reflexion of the amnion retains its relation to the amnial reflexion during subsequent rotation and develop-

ment. Its position is therefore constant at the anterior boundary of the umbilical opening where it forms the "anterior mesodermal field". It is composed of secondary and primary mesoderm. The secondary element is the fusion, in front of the buccopharyngeal membrane, of the mesodermal wings situate on both sides of the axial structures. The primary element is the blending of the amnial and yolk-sac mesoderm at the anterior pole of the blastoderm. In its mode of formation the anterior mesodermal field bears comparison with the posterior mesodermal field bounding the umbilical opening caudally where one also finds a fusion of the primary and secondary mesoderm distal to the cloacal membrane.

In this embryo the pericardial plate is placed ventral to the head fold and cranial to the buccopharyngeal membrane. Dorsally it is covered by ectoderm, ventrally by endoderm; and its anterior and lateral boundaries are mesodermal,



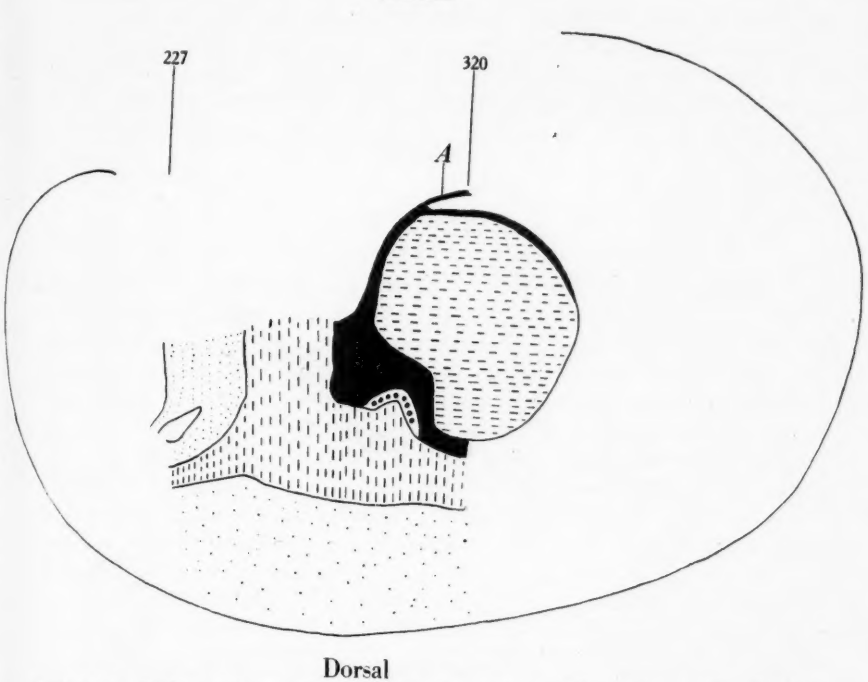
Text-fig. 1. Embryo McIntyre I. Reconstruction of a median sagittal section. × 100. The numbers refer to the sections. Description in text. Interrupted horizontal lines = ectoderm. Interrupted vertical lines = head process and prochordal area. Crosses = buccopharyngeal membrane. Black = mesoderm.

the anterior mesodermal field. In the cranial portion of the disc there are small mesodermal cavities. In the human embryo it seems more probable that the pericardial cavity is the result of confluence of a number of small mesodermal spaces rather than the fusion of two lateral cavities, a bilateral origin which would predestinate a ventral mesocardium, of the existence of which there is little evidence in the human embryo.

EMBRYO 2·6 MM.

At a later stage (Text-fig. 2) inversion of the pericardium is in progress, and it has rotated round a transverse axis to slightly over 90°. The ectodermal covering now occupies the anterior and antero-ventral aspects while the endodermal boundary—the floor of the foregut—is dorso-caudal but separated

from the dorsal wall of the pericardium in the median plane by a considerable thickness of embryonal mesenchyme. There has been proliferation of this tissue, i.e. the splanchnopleural mesoderm on the floor of the gut (Pl. I, fig. 3). The mesodermal field, at an early stage forming the anterior pericardial wall, is situated ventro-caudally so that in this embryo the pericardium is covered on the dorso-caudal and caudal half of the ventral aspect by mesoderm forming a continuous sheet in the midline. Laterally this mesoderm forms the



Text-fig. 2. Embryo 2·6 mm. Reconstruction of a median sagittal section. ×50. Numbers refer to the sections. Description in text. The yolk-sac is not shown. Interrupted horizontal lines = pericardial cavity. Interrupted vertical lines = gut. Large dots = liver. Black = mesoderm. *A* = amnion.

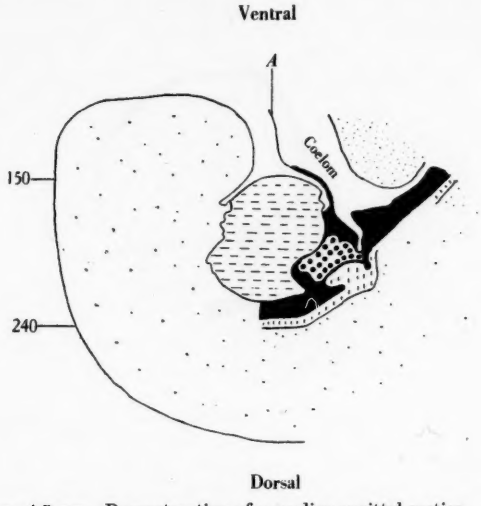
ventral wall of the coelomic tubes—communication between pericardial and abdominal coeloms. Compared with the earlier stage the area of yolk-sac connexion is reduced to the relatively small gut portal which cranially is separated from the amnial attachment by the tissue of the mesodermal field—a superior boundary of the umbilical opening—and has on each side the communication of intra and extra embryonic coeloms.

Into the proliferated mesoderm of the floor of the foregut projects the

developing liver, not as a grooved plate or gutter but as a definite hollow outgrowth of the foregut in relation to which the more deeply staining liver cells can be distinguished (Pl. I, fig. 4). A very similar hepatic diverticulum is shown in the reconstruction of the Thompson embryo of twenty-three paired somites (Keibel and Mall, Fig. 236). With the growth of the ectoderm the lateral mesodermal walls of the pericardium are taken into the body of the embryo.

EMBRYO 4·5 MM.

In an embryo of 4·5 mm. (Text-fig. 3) there has been a further rotation of the pericardium; and the cranial attachment of the amnion, carried round from the ventral to the caudal aspect, brings with it the region of the anterior mesodermal field. This now definitely forms the caudal boundary of the



Text-fig. 3. Embryo 4·5 mm. Reconstruction of a median sagittal section. ×25. The numbers refer to the sections. Description in text. The yolk-sac is not shown. Interrupted horizontal lines = pericardial cavity. Interrupted vertical lines = gut. Large dots = liver. Black = mesoderm. *A* = amnion.

pericardium and in the midline is continuous with the splanchnopleure on the floor of the foregut, the two together forming a mass of mesoderm out of which is differentiated a portion of the diaphragm and the supra-umbilical abdominal wall. Following the completion of pericardial rotation the amnion becomes the cranial boundary of the umbilical coelom (Pl. II, fig. 5). Cords of liver cells invade the dorsal portion of the mesodermal mass (Pl. II, fig. 6), and these are broken up by the vitelline veins. At this stage the liver is confined to the dorsal and dorso-caudal mesoderm and has not yet penetrated the ventral half of the mesoderm on the caudal pericardial wall.

The umbilical veins traverse the mesodermal mass laterally on each side to reach the sinus venosus. They are ventral to the pleuro-pericardial openings and are not broken up by the liver cells.

In the interests of convenience the familiar term septum transversum will be used here as denoting the general mass of mesoderm, caudal and dorso-caudal to the pericardium, i.e. including the anterior mesodermal field and the proliferated splanchnopleure of the floor of the foregut, but not confined to the unsplit cranial pericardial rim which according to Frazer forms the septum transversum after rotation of the heart.

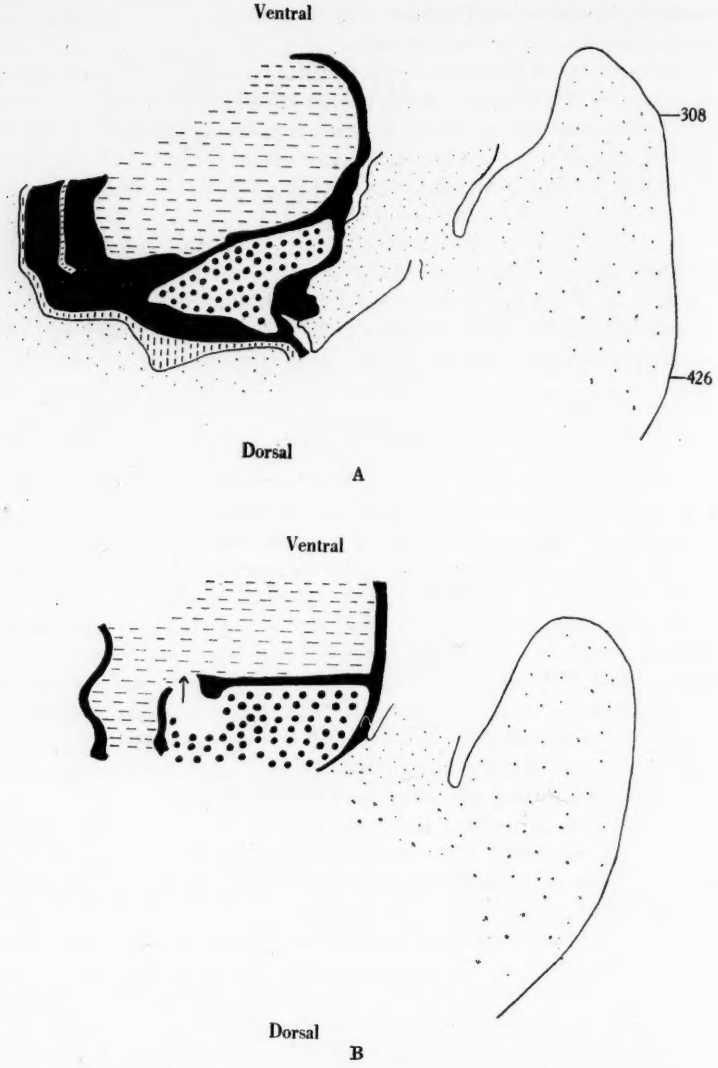
As this "septum transversum" is but part of the general body mesoderm, merging laterally with the somatopleure and in the midline with the splanchnopleure, it is in many ways unfortunate that it has been singled out by a distinctive name. This tends to isolate it as an anatomical entity and causes considerable confusion when any endeavour is made to trace its ultimate fate as such.

EMBRYO 7 MM.

In a 7 mm. embryo (Text-fig. 4A) the liver cells, which in the 4·5 mm. embryo are confined to the more dorsal part of the septum transversum, have now extended ventrally and invaded the region of the septum on the caudal pericardial wall formed from the anterior mesodermal field. Pl. II, fig. 7 is a section through the cranial attachment of what is now the umbilical cord, and it is evident that this is lying some distance caudal to the pericardium, i.e. there is in this embryo a small interval between the umbilical cord and the pericardium. This specimen is the first of the series in which an umbilical "cord" as such can be said to exist, and with its completed formation there appears a supra-umbilical abdominal wall whose mesodermal basis is the ventral-caudal part of the septum transversum, i.e. the anterior mesodermal field pushed caudally and ventrally by the developing liver.

Dorsal to the cranial attachment of the cord the mesoderm of the "septum" blends with the more myxomatous tissue of the cord, the two together forming a partition between the intra and extra umbilical coeloms (Text-fig. 4A and Pl. II, fig. 8).

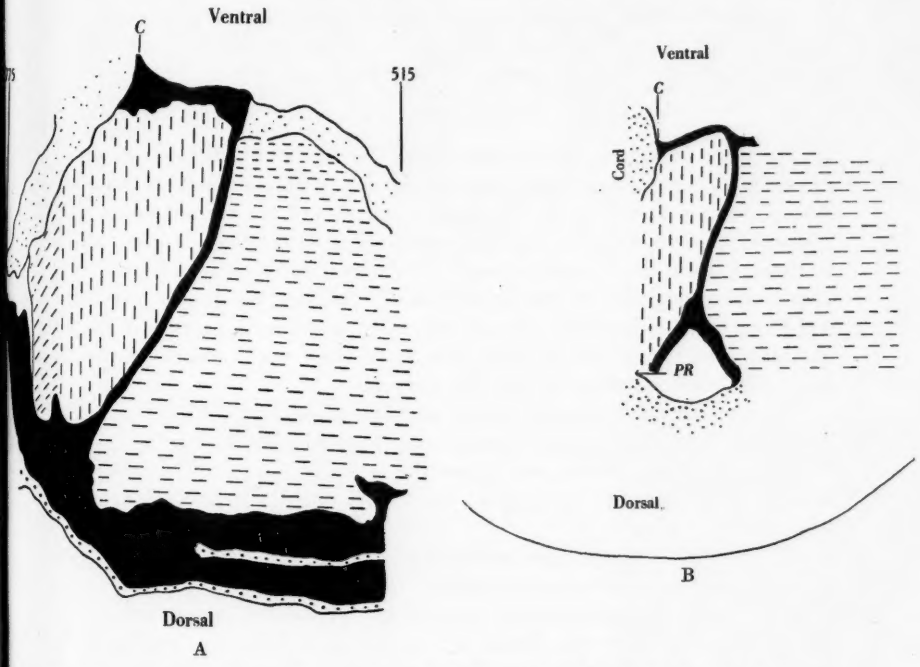
At this stage the mesodermal mass of the septum transversum is divided by the invading liver into a cranial portion, separating the liver from the pericardium, and a ventro-caudal portion—the mesodermal basis of the supra-umbilical wall. In the midline dorsally is the ventral mesentery of the foregut, and on each side of the ventral mesentery the lung buds occupy the pericardio-peritoneal channels or coelomic tubes whose ventral walls—the dorsal part of the septum transversum—form a pleuro-peritoneal membrane (Pl. III, fig. 9 and Text-fig. 4B).



Text-fig. 4. Embryo 7 mm. The numbers refer to the sections. Description in text. A. Reconstruction of a median sagittal section. ×25. B. Reconstruction of a parasagittal section. ×25. Interrupted horizontal lines = pericardial cavity and coelomic passage. Interrupted vertical lines = gut. Large dots = liver. Black = mesoderm. Oblique interrupted lines = trachea. Arrow indicates the junction of the ductus venosus with the sinus venosus.

EMBRYO 12·5 MM.

The interval between the caudal margin of the pericardium and the cranial attachment of the umbilical cord increases until in an embryo of 12·5 mm. it measures 0·6 mm. in length (Text-fig. 5A). Its mesodermal basis consists for the most part of a narrow band of tissue of loose texture whose dorso-ventral depth increases caudally. In the midline the mesoderm is denser compared to the more open mesenchymal network of the cord tissue (Pl. III, fig. 10).



Text-fig. 5. Embryo 12·5 mm. The numbers refer to the sections. Description in text. A. Reconstruction of a median sagittal section. ×25. B. Reconstruction of a parasagittal section. ×12. Interrupted horizontal lines = pericardial cavity. Interrupted vertical lines = liver. Large dots = gut and trachea. Black = mesoderm. *C* = cranial attachment of umbilical cord. *PR* = pleural cavity. Arrow indicates the pleuro-peritoneal opening.

Along with the increased supra-umbilical interval is the marked caudal and ventral growth of the liver carrying with it the tissue of the septum transversum, the mesodermal basis of the supra-umbilical wall. In this embryo the "septum transversum" is, in the midline, represented by the mesoderm of the supra-umbilical abdominal wall, the narrow strip of mesoderm (Text-fig. 5A) between the liver and pericardium which blends with the ventral

mesentery of the foregut, and in the more cranial sections extends the entire width of the body cavity (Pl. III, fig. 11), the pericardio-peritoneal membrane.

Caudally the thoraco-abdominal partition is completed on each side by the pleuro-peritoneal membranes which with the expansion of the lungs have extended at the expense of the somatopleure. The somatopleure also furnishes the pericardio-pleural membrane (Pl. III, fig. 12). Text-fig. 5B shows the continuity of these three mesodermal septa with one another and with the supra-umbilical portion of the anterior abdominal wall.

The liver has commenced to separate from its mesodermal bed, the process of "peritonealization" (Frazer), but still maintains a broad connexion ventrally where the vitello-umbilical vein enters the liver lateral to the midline, and also dorsally where the ductus venosus enters the sinus venosus.

CONCLUSIONS

The older embryologists postulated the formation of skin plates in connexion with the somites, these passing ventrally with the paraxial muscular downgrowths to complete the ectodermal covering of the ventral body wall. The more modern conception of the expansion of the embryonic body round a relatively fixed rim or circumference—the future umbilical orifice—not only affords an explanation of the formation of head and tail folds, pericardial inversion, and the prevailing dorsal convexity, but is also supported by the constant position of the umbilical vein along the "rim", and which in the later stages encroaches on the midline ventrally.

The mesodermal basis of the lateral portions of the ventral wall is of course, in the first instance, the somatopleure, reinforced later by the muscular sheets of paraxial origin. Above and below the umbilicus are areas of fusion of primary and secondary mesoderm—the anterior and posterior mesodermal fields.

The mesoderm of the supra-umbilical abdominal wall is the ventral portion of the general mass forming a partition between the thoracic and abdominal cavities, and its splanchnopleure becomes the falciform ligament and serous coat of the liver. Into the dorsal part of the mesodermal partition projects the early hepatic bud, while later the liver not only encroaches on the ventral part, but in its further growth appears actually to push before it the abdominal parietes.

The appearance of a supra-umbilical interval synchronizes with the ventral and caudal growth of the liver tissue into the mesoderm of the body wall, and one is tempted to assume the interdependence of cause and effect. Certainly the growth of this part of the abdominal wall follows on the enlargement of the liver but only provided other conditions are normal—the cause is not always followed by the effect. In congenital abnormalities involving deficiency, partial or complete, of the supra-umbilical wall, e.g. abdominal fissure or eventration, there may be a liver normal in size and shape.

The development of the abdominal coverings would therefore seem to some

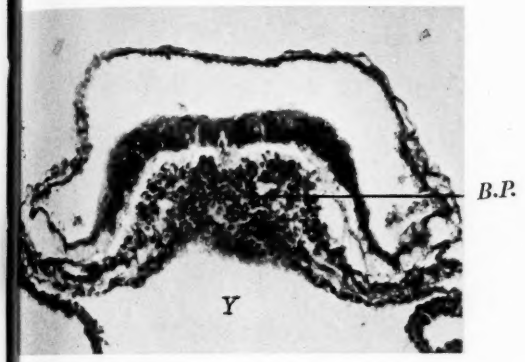


Fig. 1.

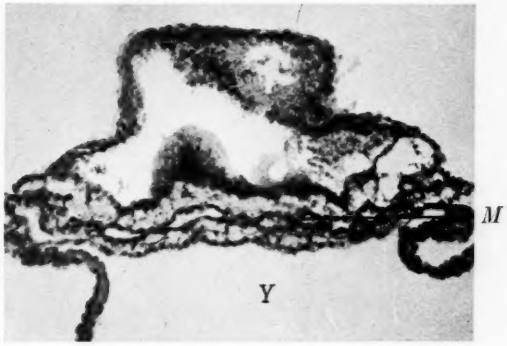


Fig. 2.

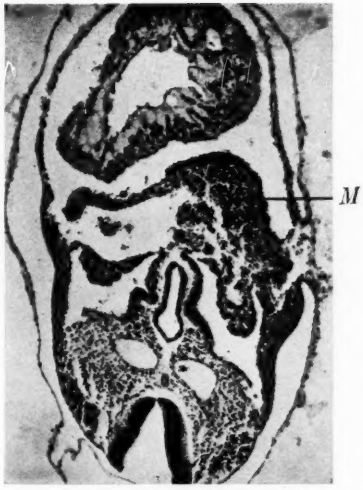


Fig. 3.

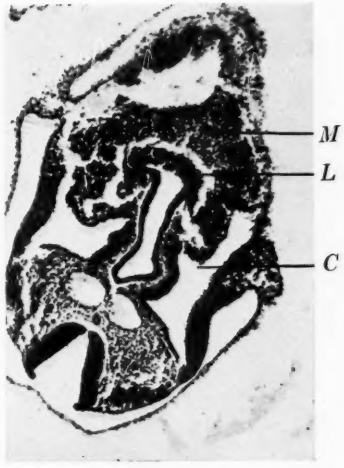


Fig. 4.

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Fig. 5.

Fig. 6.

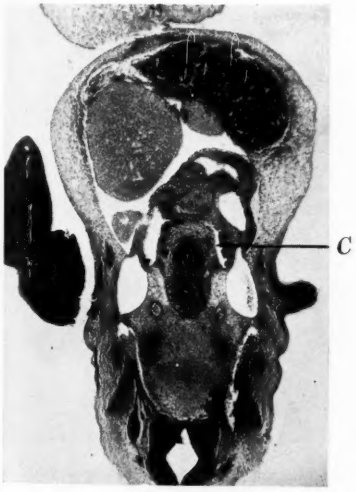

Fig. 7.

Fig. 8.

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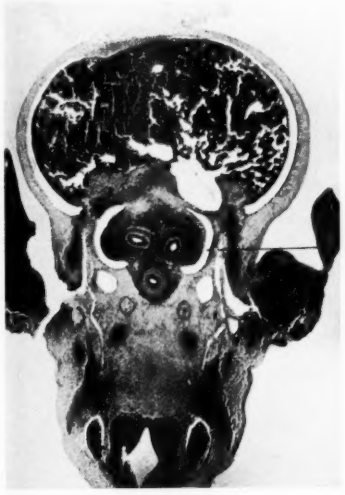

Fig. 9.

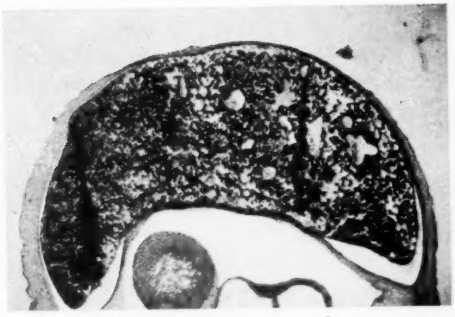

Fig. 10.

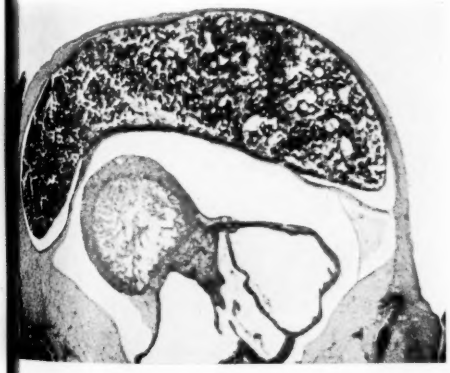

Fig. 11.

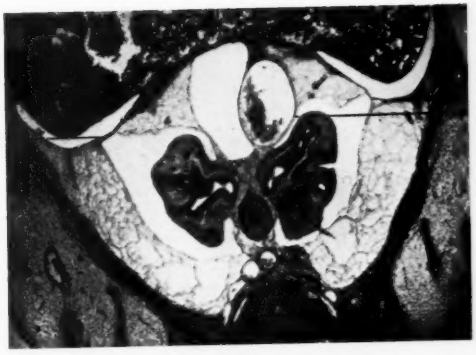

Fig. 12.

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extent to be independent of the viscera and influenced by factors mechanical or otherwise which do not necessarily affect visceral growth.

From the study of this series of embryos one is of the opinion that the name septum transversum, if it must be utilized, should have a much wider and more generous interpretation than it enjoys at present. In the 7 mm embryo the pleuro-peritoneal membrane is the mesoderm of the ventral wall of the coelomic tubes, and with thoracic expansion mesoderm from the somatopleure enters into its formation, but it would clearly be impossible at any given stage to delimit the tissue of an original septum transversum. This would indicate that the only justifiable embryological division of the diaphragm is on the basis of its muscular morphology.

SUMMARY

1. The development of the supra-umbilical abdominal wall has been studied in a series of embryos ranging from 1·4 to 12·5 mm.

2. The ventral portion of the mesodermal partition between the thoraco-abdominal cavities furnishes the basis of the supra-umbilical wall.

3. This ventral part of the thoraco-abdominal partition is itself derived from the anterior mesodermal field subsequent to pericardial inversion.

4. The term "septum transversum" is criticized as being misleading and the cause of much confusion in the embryological interpretation of the diaphragm.

I have to record my indebtedness to my chief, Prof. Blair, for his supervision of the work.

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EXPLANATION OF PLATES I–III

Plate I

Figs. 1, 2. Photomicrographs of embryo McIntyre I. ×*c.* 114. Description in text.

Figs. 3, 4. Photomicrographs of 2·4 mm. embryo. ×*c.* 54. Description in text. *Y* = cavity of yolk-sac; *B.P.* = buccopharyngeal membrane; *M* = mesoderm; *C* = coelomic passage; *L* = liver cells.

Plate II

Figs. 5, 6. Photomicrographs of section of embryo 4·5 mm. ×*c.* 28. Description in text.

Figs. 7, 8. Photomicrographs of section of embryo 7 mm. ×*c.* 20. Description in text. *A* = amnion; *M.L.* = mesoderm with liver cells; *C* = coelomic passage; *M* = mesoderm; *P* = pleuro-peritoneal opening.

Plate III

Fig. 9. Photomicrograph of embryo 7 mm. × *c.* 20. Description in text.

Figs. 10–12. Photomicrographs of embryo 12·5 mm. ×*c.* 21. *C* = coelomic passage; *P.P.* = pleuro-peritoneal membrane; *P.D.* = pleuro-pericardial membrane.

THE INVESTIGATION OF CELL SIZE WITH SPECIAL REFERENCE TO THE HUMAN LYMPHOCYTE[1]

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THE VALUE AND INTERPRETATION OF CELL SIZE MEASUREMENTS

From time to time reports of work on cell size are published, but the interest in the subject is not nearly so great as at the beginning of this century. Many observers feel that cell measurement is an unprofitable type of work, in spite of the fact that certain sizes and size differences in cells or parts of cells have been shown to have value even in clinical medicine and animal pathology, as is exemplified by the work of Price-Jones (1933) on human red blood cells; of Savage and his collaborators (1927, 1930) who showed that the head sizes of spermatozoa in bulls and horses can be used to indicate sterility; and of Quensel, whose ratio (nucleolus : nucleus) is being used in the identification of malignant cells (Foot, 1937). In pure biology it should surely be obvious that, if analysis of cell structure or mechanism is to be made in terms of physics and chemistry, such analysis must be largely quantitative and must involve, among other things, the consideration of cell sizes. Current research reveals in some instances an unreasonable scepticism towards cell size determinations, and in other instances a facile acceptance of size differences in histological preparations as indicating size differences in the original tissues. It is difficult to conceive of a method being found whereby one may determine the actual sizes of healthy cells in situ in animals or plants, except in those that are unicellular or have few cells; and it must never be forgotten that cells in histological preparations such as sections or blood films are merely the much altered "skeletal remains" of the original cells. Even cells from tissue cultures cannot be accepted as equivalent to those of the tissues from which they arose. These facts do not, however, justify complete scepticism regarding the value of cell size in pure or applied cytology. Methods of blood counting, of cardiac percussion and of many other quantitative clinical tests have an unknown degree of bias (systematic error) which does not prevent their use in differential diagnosis. Similarly, cell sizes in films or histological sections can be used without knowledge of their relationship to the original cell sizes. In such procedures, however, this lack of knowledge must not be overlooked. In a collection of sections of carcinoma from the mucosa of the body of the

[1] This research was conducted with the aid of a grant made to one of the authors (B. K. C.) by the Banting Research Foundation, Toronto, Canada.

human uterus the nuclear sizes were found to be greater than in specimens of non-malignant hyperplasia fixed and embedded in the same way, and a similar difference was found in another collection of specimens prepared by a different method (Mainland, 1933). The obvious conclusion that the nuclei in situ had differed in the same way as in the sections is not permissible. The results might be attributed to differences in nuclear structure, or in the surrounding fluids, that caused the cancerous nuclei to alter during the histological preparation in a different way or to a different degree from the nuclei in hyperplasia.

Various studies have been made by one of us (D. M.) of nuclear and cell size in histological sections and blood films, normal and pathological, partly to ascertain the possible diagnostic value of such determinations and partly as an approach to the more fundamental question of size relationships between unaltered cells in human tissues. The next step in the latter direction is naturally the experimentation with human blood cells which can be isolated from the body without grave injury to them. Previous study of human blood films (Mainland *et al.* 1935) showed that variation in lymphocyte sizes between patients of different ages, of both sexes, and with various diseases was not appreciably greater than the variation in lymphocyte sizes between films made, even on the same occasion, from the blood of one healthy person. This naturally led to the exploration of possible causes of the variation in and between films, and comparison with preparations of lymphocytes in isotonic saline. This exploration is the subject of the present paper. The technique, analysis and interpretation of the results are discussed in considerable detail because the same or similar problems arise in any investigation of cell or nuclear size. Tests and statistical quantities are mostly referred to by name only; for example, the standard deviation, standard error, analysis of variance, correlation, regression, and Fisher's z and t tests for small samples. The methods used were those given by Fisher (1935, 1936), which are further exemplified in their application to biological problems by Snedecor (1934) and Goulden (1936). Attention is commonly paid in this paper to the meaning and interpretation of the tests rather than to their numerical results. According to the usual convention, a difference or other quantity is said to be "significant" if it would occur by chance in less than 5 % of trials, and "highly significant" if it would occur by chance in less than 1 % of trials. Although some of the investigations involve small samples or single experiments, care is taken to avoid unwarranted deductions from them. Readers who are somewhat sceptical of small sample technique and who are unfamiliar with the work of Fisher may find answers to some of their difficulties in the discussion of this problem in another connexion (Mainland, 1936 and 1937).[1] Sometimes in the present investigation odds and probabilities were found by the very simple method

[1] The subject is discussed at greater length by the senior author (D.M.) elsewhere (*The Treatment of Clinical and Laboratory Data.* Edinburgh: Oliver and Boyd, in the Press).

of consulting Warwick's (1932) tables as indicated in the article just referred to (Mainland, 1936).

It is beyond our present purpose to consider the literature on cell measurement. A useful review of work on unicellular organisms is given by Adolph (1931). The ten articles by Wermel and his collaborators (1932–4) report extensive observations on cell size in mammals and other animals in pathological and toxic conditions, but show a too ready acceptance of histological preparations as indicating size relationships and differences in the tissues before preparation. These articles illustrate also another important source of fallacy—that of inadequate numbers of control animals. In the investigations such as Wermel carried out, a very large number of cell measurements, even thoroughly analysed, from one animal will not make up for the lack of other animals.[1] Among recent work on lymphocytes, Wiseman's (1931) dismissal of quantitative methods after a few observations, inadequately controlled and analysed, illustrates a not uncommon attitude to quantitative methods. However much an observer may be justified by preference for qualitative methods, such a dismissal of quantitative methods cannot claim to be based on sound evidence.

THE VARIATION IN LYMPHOCYTE SIZE BETWEEN FILMS

The first series of experiments was designed chiefly to ascertain how far controllable factors might account for the great variation already referred to between mean lymphocyte sizes in different films. The drops of blood were taken from the finger of one healthy young adult male subject, and drawn along one slide by the edge of another slide. The punctures are differentiated in Tables I and II by letters. The first drop to emerge from each puncture was discarded as in blood counting, and the subsequent drops were numbered in sequence. Unless otherwise stated the films were treated by the "control" method, that is, they were dried in the air, and covered by Wright's blood stain for 2 min., then by Wright's stain and distilled water in equal parts for 2 more min., and washed in distilled water usually for from 15 to 30 sec. As described in more detail elsewhere (Mainland *et al.* 1935), the images of the cells were projected on to white cardboard and traced by pencil at a linear magnification of approximately 1800.

Incorrect estimation of the magnification is, as Ponder (1934) pointed out for red blood cells, a serious source of discrepancy between the records of different observers. In the present observations the coarseness of the lines of the stage micrometer projected at such a high magnification precludes great certainty regarding the magnification, but in no way vitiates the comparisons made within the series of experiments conducted with the same equipment. A spurious suggestion of accuracy is avoided by expressing the results in mm.,

[1] In the present paper the experiments on technique are such that the blood of one person can give valid results; and it will be pointed out where investigation of other persons' blood s necessary as a further step.

the units in which the tracings were measured, but here and there a rough conversion into μ is made.

Throughout the observations in this paper the sizes are expressed as average diameters, the long axis (length) of each cell being added to the transverse axis (breadth) at right angles to it, and the sum divided by two. This method was chosen because the lymphocyte investigation commenced as a comparison with Price-Jones's red cell diameters estimated in the same way. It is not necessarily the best expression of cell size, and further exploration will be concerned with other expressions, including thickness measurements, many of which have been made. Although in most parts of the work nuclei were measured, they will not be discussed in this paper.

Table I. *Experiments on the preparation of blood films*

Series letter and no. of film	Experiment	No. of lympho-cytes measured	Mean diameter of lym-phocytes (mm.) on tracings	Standard devia-tion mm.	Standard error of of mean mm.	Monocytes No.	Monocytes Mean diameter mm.	Mean diameter of ten red blood cells mm.
			Drying of original film					
D1	Control (dried in air)	95	15·94	2·10	0·22	5	21·78	13·67
D2	Dried near calcium chloride	91	16·97	2·22	0·23	8	22·77	13·60
D4	Dried over flame	97	18·31	1·88	0·19	3	22·50	13·54
D5	Control	94	18·08	1·65	0·17	6	24·60	13·91
			Drying after staining					
FD1	Control (dried in air)	96	15·49	2·26	0·23	4	23·10	13·17
FD2	Dried near calcium chloride	94	18·45	2·01	0·21	6	24·38	13·97
FD3	Dried near water	94	16·34	1·84	0·19	6	24·95	13·83
FD4	Dried over flame	89	16·11	2·10	0·22	11	21·64	14·46
FD5	Control (dried in air)	93	16·28	1·75	0·18	7	23·36	13·87
			Blood diluted with hypotonic saline					
X1	Control	93	17·09	1·72	0·18	6	22·45	13·46
X2	Saline	98	14·50	2·34	0·23	2	22·20	13·17
X4	Control	96	18·63	1·95	0·20	3	25·62	13·54
			Blood diluted with isotonic saline					
I1	Control	90	17·88	2·15	0·23	9	24·50	13·55
I2	Saline	96	14·05	2·16	0·22	3	23·00	12·10
I3	Control	94	16·43	2·45	0·25	4	22·14	13·12
			Blood diluted with isotonic formaldehyde					
F1	Control	98	15·43	1·72	0·17	2	21·83	13·99
F3	Formaldehyde	100	9·39	1·06	0·11	0	—	9·69
F4	Control	96	17·14	2·07	0·21	4	24·06	13·86

The pairs of the control films in Tables I and II, taken from the same puncture, show significant differences in mean lymphocyte diameters, that is, the differences between the two members of a pair are greater than twice the standard error of the difference. Even the control method could not guarantee the same rapidity of drying and staining in two films, and the object of most of the tests was to exaggerate such differences in technique, in order to see

whether there was a corresponding exaggeration of the differences in lymphocyte size.

When only two controls are available, a difference has to be very great before it is significant, and further investigation showed that it was legitimate to combine the controls of Table I and the whole of Table II to form a "combined control" series of twenty-one films (see below), and this series was used in the following comparisons.

It has been previously found that the differences between lymphocyte sizes within films and between films cannot be accounted for by differences in film thickness as ascertained by the density of red cell distribution and by naked eye observation (Mainland *et al.* 1935, 1936). This can be interpreted as indicating that differences in rapidity of drying when the film is spread cannot wholly account for lymphocyte size differences. Direct experiment (Table I, Series D) showed no significant difference produced by drying a film near calcium chloride in a glass chamber, or by drying a film over the flame of a laboratory burner. Attempts to slow the rate of drying by placing the film in a chamber containing water vapour gave no measurable results, because of haemolysis.

Table II. *Experiments on staining of blood films*

Film	No. of minutes in stain	No. of minutes in stain and water (equal parts)	No. of seconds for washing in distilled water	No. of lympho-cytes measured	Mean diameter of lym-phocytes (mm.) on tracings	Monocytes		Mean diameter of ten red blood cells mm.
						No.	Mean diameter mm.	
E1	2	2	25	95	16·79	3	21·90	13·72
E2	1	1	15	81	19·99	17	23·11	13·79
E3	1	2	35	97	17·66	2	22·73	13·25
E5	2	1	30	93	16·31	5	23·51	14·09
E6	2	3	30	89	16·08	11	23·07	13·43
E10	3	1	30	88	15·36	11	22·54	14·05
E11	3	3	30	98	18·42	2	23·20	13·57
E12	2	2	25	95	17·76	4	24·01	13·77
E13	6	2	60	97	15·94	3	23·87	13·55
E15	2	2	25	100	17·24	0	—	13·84
E18	2	2	25	99	14·79	0	—	14·24

Note. Films E1–10 were from the same puncture in the finger of a young healthy adult male. Films E11–13 were from the same puncture re-opened after several minutes. Film E15 was made from another puncture in the same person 5 days after the first series, and E18 from another puncture 3 days later still.

It is conceivable that the methyl alcohol in Wright's stain might alter cell size. In Table II the standard errors of the mean lymphocyte sizes were of the order of 0·2 mm., and therefore there are significant differences between the various films even from the same puncture, but these differences, examined by analysis of variance and regression methods, revealed neither a trend in size from the earlier to the later drops, nor any relationship with staining technique. However much the stain may alter cell sizes within the first few seconds after it is added, the differences ordinarily found between films cannot

be accounted for by controllable differences in staining technique. (A few more experiments on differences in staining technique are mentioned later, and confirm this conclusion.) If the stain is responsible for differences within the film it presumably must produce size differences in virtue of some differences in constitution of the dried cells.

Differences in drying after staining (Table I, Series FD) could not account for the size differences, because the mean size even in FD2 was not significantly different from the mean of the combined control series.

A few experiments with saline of different strengths were carried out because, as a blood film dries, the fluid becomes hypertonic and, if the cell boundaries still act as semi-permeable membranes, a shrinkage of the cell should be expected. The subject was not pursued far, but one or two interesting results emerged. Repeated tests showed that for the blood under investigation the saline that gave the least haemolysis, swelling or shrinkage of the red cells was a 0·6 % solution of sodium chloride in distilled water. This appears rather low, but the fact has to be accepted. To some extent it may be explained by the exposure of the solution to air during the time necessarily occupied in mixing the saline with blood, or in preparing the saline mounts discussed later. A hypertonic saline (0·9 % sodium chloride) added to a drop of blood on a slide produced so much distortion of the cells that measurement was abandoned. A drop (Table I, Film X2) mixed with hypotonic saline (0·3 %) showed a mean lymphocyte size not significantly different from its own controls or from the mean of the combined control series, but the interesting feature is that, instead of being larger than the average, the tendency is, if anything, for it to be smaller. This may be accounted for by the factors mentioned below in connexion with the isotonic saline mount, but perhaps a more reasonable explanation is suggested by the notes made regarding this hypotonic saline film—that, whereas the smaller lymphocytes appeared normal, the larger ones were highly abnormal, their nuclei being ruptured presumably by endosmosis and their contents scattered through the cytoplasm. Such cells being omitted from the tracings, the average tended to be lower rather than higher. This appears to be a trivial point to note, but it has a distinct bearing on the use of cell measurement in pathological diagnosis. The results of cell measurement can easily be modified by rejection of cells that are too much altered, either by disease or by histological preparation, to give satisfactory measurements. Moreover, if the variation between cell measurements has to be compared with some standard, a difference can be introduced if the shape of the cells in the specimen under examination is different from those on which the standard was based, whether the difference is in the ratio of the two axes or is a localized distortion.

The drop (Table I, Film I2) mixed with isotonic saline showed a mean lymphocyte size significantly smaller than the mean for the combined control series. Various explanations are possible. The lymphocytes, drying more slowly than usual, may have been able to contract more than usual before they

became adherent. The dilution of the plasma protein may have contributed in the same way by lessening the rapidity of a firm adhesion. The cells may have been cooled more nearly to room temperature before adhering, and, as shown below, contraction can occur during this cooling. The gradually increasing hypertonicity of the saline may have had more chance than usual to cause shrinkage by exosmosis from the (unadherent) cells. How far such factors may explain differences between different films prepared in the ordinary way cannot be stated. The subject was not pursued, because the fact of immediate importance was that films prepared even with isotonic saline are not to be taken as equivalent to films prepared in the usual way.

The observation on formaldehyde fixation (Table I, Film F3) was merely incidental, because further investigation of such problems is to be undertaken. The difference from the combined control series was highly significant, and, even although based on a single observation, this is sufficient to show the great importance of paying attention to fixation in studying cell sizes.

Variation between punctures made on different days

Series D, FD, X, I and F provide from each puncture a pair of control films made and stained in exactly the same way. Analysis of variance showed that the differences in mean lymphocyte size between punctures made on different occasions, even on different days, was not significantly greater than the differences in mean lymphocyte size between the drops made from the same puncture. If there was a difference between the punctures, the between-drop variation was too great to allow it to be seen. In four out of the five series the second control film had a greater mean lymphocyte size than the first, but this is not sufficient evidence of an upward trend, for the ratio is not significantly different from a one-to-one ratio. This agrees with results from Table II discussed above.

Combined control series

The results stated in the last paragraph show that the control films of the five series mentioned there can be considered as equivalent in size variation to a series of drops made from the same puncture. For similar reasons the eleven films of Table II can be added, to make the combined control series of twenty-one films already referred to.[1] From this series the following values were estimated:

Mean of twenty-one mean lymphocyte diameters: 16·892 mm. (approx. 9·4 μ).
Standard deviation estimated from series: $\pm$1·288 mm. (approx. $\pm$0·72 μ).
Coefficient of variation: 7·64 %.

This coefficient is not appreciably different from the coefficients of variation between films obtained from patients of different ages and with different

[1] Some of the items in this control series were used in the brief survey of normal films recorded elsewhere (Mainland *et al.* 1935, p. 420).

diseases (Mainland *et al.* 1935), that is, 9·46 % for twenty-five males, and 8·78 % for thirty females.

This combined control series has already been used in testing the significance of differences in experimentally treated films, and a glance at the rationale of this use of the series is desirable, because such tests should never consist in the mere application of numerical rules. In this series the measurements are arranged on each side of the mean, few being situated at a relatively great distance above or below. As in most such investigations in biology, where no one constant major factor can be detected as responsible for the variation, the frequency distribution tends to be of the "cocked-hat" type, sufficiently like the (statistically) normal curve to justify one in expecting that, as for the normal curve, twice the standard deviation measured from the mean on each side will exclude roughly 5 % of the items. The 2 S.D. range in this series is $16{\cdot}892 \pm 2 \times 1{\cdot}288$, that is, from 14·316 to 19·468 mm. It excludes one out of the twenty-one items. The drop prepared with isotonic saline (Table I, Film I2) had a mean lymphocyte diameter of 14·05 mm., which is below the −2 S.D. level. Such discrepancies are greater than what is usually attributed to chance; but in this instance the total number of observations is rather small—twenty-two (the number in the control series *plus* the film that is being tested), and where such total numbers are under thirty it is desirable to apply special tests. These tests become more and more necessary as the numbers go below twenty. The comparison of means by the t test of Fisher (1936, § 24.1) still showed that the difference in this case was significant. The same test showed a highly significant difference in the formaldehyde film (F3).

The control series enables one to indicate how great a difference between a pair of films must be before it can be attributed to anything beyond the differences represented in this series. The differences in this series represent, not a change in lymphocyte size in the blood itself, but other sources of variation, for example, difference in lymphocyte sizes in the different drops as they emerge from the same puncture or as the drops are sampled, or differences that arise in the subsequent treatment, even although the technique is standardized. If the standard deviation has been estimated from a large series, the difference between two items taken at random must be $2\sqrt{2}$ times the standard deviation before it can be considered significant, and even greater allowance must be made where, as here, the series contains less than sixty items. Details need not be given, but Fisher's z method for analysis of variance and Snedecor's (1934) Table of F, constructed from Fisher's tables, show that the minimum significant difference in this instance would have to be 3·80 mm. (over 2μ if unmagnified). The fact that this is more than 20 % of the mean (16·892 mm.) shows how difficult it would be to prove that the blood itself had changed between one observation and the next.

The relationship between lymphocyte sizes and the sizes of other cells

Monocyte and red cell mean diameters, obtained as for lymphocytes, are given in Tables I and II. The coefficient of correlation between mean lymphocyte diameter and mean monocyte diameter in the control series was +0·49, a significant value. Higher values were found in series from different patients (Mainland *et al.* 1935), and it is tempting to explain this by suggesting that part of the relationship is due to technique, acting in the same way on lymphocytes and monocytes, and therefore manifested in the present series where all the drops were obtained from the same person; while part of the relationship is a primary one, whereby the different classes of leucocytes in any one person correspond to each other in size. Although decisive evidence is lacking, we are inclined to attribute the whole relationship to technique. The correlation between lymphocyte size and mean red cell size in the control series had the non-significant value of −0·176; in contrast to the significant values (+0·54 and +0·69) in the observations on different patients just referred to.

SIZE DIFFERENCES IN THE ARRANGEMENT OF LYMPHOCYTES IN FILMS

The experiments described above, although limited, have indicated that something more than rate of drying and controllable differences in staining technique must be looked for to explain the lymphocyte size differences between and within films. A suggestion of such a factor has already been obtained (Mainland *et al.* 1936). In one film all the lymphocytes were measured and it was found that larger lymphocytes were more at the periphery, while the smaller were more centrally placed. Other films less completely examined agreed with this, and, although the evidence was not sufficient to prove that such an arrangement is likely to be universal, it agrees so closely with Gyllenswärd's (1929) demonstration of the distribution of leucocyte classes according to relative sizes, that one can combine the two pieces of information as evidence that, whatever may be the detailed arrangement or mechanism, the distribution of leucocytes in films is related to their size. Fallacies in the interpretation were considered and eliminated in the paper referred to (Mainland *et al.* 1936). Further information is provided by the following experiment. Each of 15 drops of blood taken from the same healthy young adult male subject was spread between two square cover-glasses, an upper and a lower; all films were stained by Wright's stain and mounted on a slide with balsam, the upper and lower film of each drop being side by side on the same slide. The duration of exposure to pure stain and to half-strength stain was in some drops purposely made different between the two films, upper and lower. The time differences were recorded, but there was no discoverable relationship between the differences in staining and the size differences noted below. The rather complicated details of these controlled staining experiments

can therefore be omitted. On the top of each cover-glass near the centre of the film a small ink square was outlined in such a position that the thickness of the part of the film within the square was, for each member of any pair of films, as nearly equal as could be judged by the naked eye in a good light. This was a precaution against the possibility of attributing size differences to differences in the rate of drying, as indicated by differences in thickness of films. It was supplemented by a further test described below. The measurements were made by a Zeiss filar screw eyepiece micrometer, an oil immersion objective being used. From each of the ink squares ten lymphocytes were measured (two long and two short axes on each). The mean of the ten measurements in micrometer units is shown in Table III. In only one of the fifteen pairs was the mean lymphocyte size greater on the upper film, and, even without any special test of the numerical values of the differences, it can be shown,

Table III. *Comparison of lymphocyte sizes on upper and lower cover-glasses*

(Each figure is the average, for ten cells, of the mean diameters, $\frac{\text{length} + \text{breadth}}{2}$, in eyepiece micrometer units.)

Drop no. ...	U11	U14	U24	U25	U26	U30	U31	U32
Upper film	31·76	38·49	36·41	31·40	35·69	36·58	30·39	36·98
Lower film	37·03	38·39	40·05	35·44	37·06	39·29	34·71	37·85
Difference (lower *minus* upper)	5·27	−0·10	3·64	4·04	1·37	2·71	4·32	0·87
Drop no. ...	U33	U34	U35	U36	U37	U38	U39	
Upper film	36·70	34·31	35·61	36·00	31·78	35·10	37·35	
Lower film	36·73	35·66	37·36	37·25	36·50	36·19	37·88	
Difference (lower *minus* upper)	0·03	1·35	1·75	1·25	4·72	1·09	0·53	

for example from Warwick's (1932) probability tables, that such a difference from one-to-one ratio would occur by chance only once in a thousand times if there were no special tendency for either lower or upper films to have larger lymphocytes. Even although this method obviously gives undue weight to the small negative difference, it proves the high significance of the difference. The test involving the numerical values of the differences gives due weight to each, as follows:

Mean of fifteen differences: 2·189 micrometer units.
Standard deviation estimated from the sample of fifteen: ±1·779 units.
Standard error of mean: ±0·459 unit.

The mean is 4·77 times its standard error and therefore very highly significant, even when allowance is made for the small size of the sample by the *t* test (Fisher, 1936, § 24). The difference is about 6 % of the mean diameter (36·065 units) calculated from the thirty films. As estimated from a stage micrometer each unit in the eyepiece micrometer represented 0·255 μ. The mean difference is, according to this estimate of the conversion factor, 0·56 μ.

As a further test of the possibility that film thickness was responsible for these differences, a paper with a square opening of about ½ mm. side was inserted into a 10 × eyepiece and each of the ink squares in which lymphocytes had been measured was surveyed under high power. The number of red cells, varying from forty to one hundred, that appeared in the paper square in the eyepiece was counted at sixteen different parts of each inked square, the points of survey being evenly distributed throughout the inked square with the aid of the scales on the mechanical stage of the microscope. There was no suggestion of a relationship between the difference in lymphocyte sizes in the upper and lower films and the density of distribution of the red cells, which is an indication of film thickness.

However readily one may admit that the preparation of the films is likely to change cell sizes, it seems difficult to account for the larger mean size on the lower films except by supposing that, on the average, the lymphocytes that are relatively larger before treatment are relatively larger on the films. This does not suggest the maintenance of any exact size relationship, and yet it is in agreement with the facts mentioned above regarding size distribution of lymphocytes and of other leucocyte classes in films prepared on slides instead of cover-glasses. Although the forces of capillary attraction, gravity, resistance of neighbouring cells and of surrounding fluid must interact in a very complicated way on individual cells, a relatively simple physical explanation can account for the average difference—the predominance of larger cells on the lower films. The mass of a cell, and therefore the gravitational pull on it, must be, at least approximately, proportional to its volume. The frictional resistance will be proportional to its surface area. Since the volume of a sphere is proportional to the cube of its radius, and the surface area is proportional to the square of its radius, the larger cells will present, in proportion to their volume, a relatively smaller surface to frictional resistance, and the resultant downward pull will be greater. Consequently the acceleration will be greater and they will reach the lower film more quickly and in relatively greater numbers than do the smaller cells.

The physical explanation just given may well be over-simplified, but important consequences must arise from the fact that measurable differences in size distribution can be, however incompletely, attributed to such physical forces and to associated phenomena such as the relationship of mass and momentum discussed elsewhere (Mainland *et al.* 1936) as a factor in irregular size distribution in blood films made on slides. Such physical forces must be at work wherever drops of liquid such as blood, suspensions of bacteria, or fluids from tissue cultures are taken as samples for the measuring or counting of cells, and if they produce measurable bias, as they apparently have done in the present experiment, they may well be partly responsible for the measurable differences in other instances. They may be important causes of the differences in lymphocyte sizes between drops from the same puncture. Now if the degree of this bias were constant from specimen to specimen taken

under the same conditions, it would probably present no serious obstacle; but it is difficult to make the conditions sufficiently uniform. Even if the size of the drop of blood (or other fluid) and the speed of film spreading were always the same, and even if all the cells in each film were measured, uncontrollable variation would remain. For example, the physical forces discussed above must affect blood cells as they emerge from a punctured finger and as the drop is being lifted by the cover-glass or slide. If these forces produce a bias which differs in degree from one sample to another, samples taken under the most carefully controlled conditions will vary from each other more than chance can account for, that is, more than would samples taken purely at random—samples in which each cell has an equal chance of inclusion. Even if every cell emerging from a puncture had not an equal chance of being included in the sample or of being measured, the variation between samples should not be greater than chance could account for, if the degree of bias in favour of or against cells of certain sizes were constant. The variation in lymphocyte sizes between samples (films) is very commonly greater than chance could account for, and the next step is to find out whether such variation can be reduced by measuring the cells under conditions more like the original conditions, that is, in saline. If the variation can be reduced, even if the bias cannot be wholly removed, this technique will be more suitable for the demonstration of size differences between the lymphocytes of different persons or of the same person under different conditions.

MEASUREMENT OF LYMPHOCYTES IN SALINE

The blood from finger punctures in the same healthy young adult male was used throughout the investigation. The dilution was made in a Trenner red cell pipette with automatic capillary volume adjustment, diluting 1–200, the diluting fluid being the 0·6 % sodium chloride in distilled water which, as discussed above (p. 379), was found to be isotonic for this blood. The saline was tinted with gentian violet so as to stain the nuclei. There is a certain objection to this method because the staining of the nuclei may well indicate that the cells have been so acted on by the stain as to be moribund or perhaps dead. One of the objects was, however, to use a simple method that could be applied in conjunction with clinical observations and which would give a good nuclear stain; not necessarily a method that would give the closest possible approximation to the conditions in the blood. Extensive observations with supravital stains have therefore been delayed.

All solutions, pipettes and haemocytometers were kept in an incubator at body temperature (37° C.) before and after use. Before a drop of liquid was expelled from a pipette the latter was shaken by hand for 3 min.

The difficulty of obtaining sufficient working distance and avoiding pressure on the cells during measurement was overcome by mounting the drops of

diluted blood on the plateau of a Levy-Hausser haemocytometer counting chamber of depth 0·1 mm.; covering the drop with a Hausser reinforced cover-glass (transparent area 12 × 14 mm. and thickness 0·18 mm.); and using a Zeiss oil immersion objective (× 50, N.A. 0·85) with a free working distance of 0·40 mm. (The haemocytometer and cover-glass had United States Bureau of Standards certificates of accuracy.) Cover-glass clips were used to reduce the tendency for the immersion oil to lift up the cover-glass during focusing. The measurements (two lengths and two breadths for each cell) were made by a Zeiss filar screw eyepiece micrometer. One division of the micrometer was estimated as equivalent to 0·236 μ, but for the most part the readings are given in micrometer units.

Temperature and lymphocyte size

It might naturally be supposed that cell size would differ appreciably at room temperature and body temperature in warm-blooded animals, and it is rather surprising that this point is often overlooked in studies of cell size. The question in the present investigation is: how far can the variation in lymphocyte sizes between films be accounted for by differences in the extent to which different cells and different films cool before they are dry and fixed? To determine how much change in size can be associated with cooling of lymphocytes, the microscope with which the examinations were made was placed in a wooden box containing a 16 C.P. carbon filament lamp and a metal filament lamp used also as illumination for the microscope. Each lymphocyte, measured in saline as above described, was taken from a separate puncture, and for each lymphocyte three sets of measurements were made: at body temperature (37° C.); at room temperature (24–27° C.) attained by turning off the heating lamps; and at a low temperature, recorded in Table IV, attained by placing carbon dioxide snow on copper troughs on the microscope stage. The temperatures given are those recorded by a thermometer with its bulb resting on the haemocytometer very near the objective, and although they doubtless do not accurately represent the temperature of the cells, the recorded differences in temperature are sufficient for the present purpose. From about 10 to 25 min. were required for each of the two cooling processes. The time taken by the measurements was about 3 or 4 min. at each temperature. The variation of temperature during the body temperature measurements was commonly from one- to three-tenths of a degree, slightly more at room temperature, and at the low temperatures sometimes 2, 3 or 4 degrees. The results are shown in Table IV, where the diameters are the means of the two length and two breadth measurements, and the temperatures are the means of the readings taken at the beginning and end of the four measurements.

In all except one out of the twenty-six lymphocytes the mean diameter at room temperature was lower than at body temperature, and the small

apparent increase in cell 15 could easily be accounted for by error in measurement. The following estimates were made:

Mean decrease for the twenty-six lymphocytes: 1·807 micrometer units (about 0·43 μ).

Standard deviation of the series of differences: $\pm$1·625 units.

Standard error of mean: $\pm$0·319 unit.

The mean is between five and six times its standard error, and therefore highly significant. It is about 4·2 % of the mean diameter of the twenty-six cells at

Table IV. *Alteration of lymphocyte size during fall of temperature*

(The sizes are mean diameters, $\frac{\text{length}+\text{breadth}}{2}$, in eyepiece micrometer units.)

Lympho-cyte no.	Mean diameter at body temperature	Mean diameter at body temperature *minus* mean diameter at room temperature	Low temperature (° C.)	Mean diameter at room temperature *minus* mean diameter at low temperature
1	42·550	0·375	12·25	1·625
2	38·375	3·125	9·50	−0·450
3	41·650	0·900	9·10	0·425
4	43·875	0·375	9·15	−0·875
5	45·875	2·200	11·05	0·400
6	39·350	0·100	11·40	3·375
7	39·725	0·850	7·90	0·125
8	43·025	0·350	9·40	0·500
9	46·350	0·075	9·70	−0·200
10	45·625	1·125	8·25	6·575
11	46·875	2·675	8·00	3·250
12	48·950	1·125	8·90	2·600
13	49·400	7·050	4·20	−4·400
14	37·575	0·550	9·50	0·925
15	41·425	−0·700	10·80	0·075
16	43·225	0·950	10·95	−3·525
17	40·850	1·550	8·90	1·125
18	43·950	4·375	7·45	−0·350
19	44·175	2·750	8·20	0·475
20	47·900	3·375	10·85	0·475
21	40·300	2·775	9·55	0·625
22	41·650	2·425	6·60	0·600
23	39·200	2·725	7·40	−0·825
24	41·725	2·325	7·80	0·050
25	43·425	1·675	11·85	1·325
26	40·400	1·875	3·90	1·225

body temperature. This difference cannot well be attributed to the standing of the cells, perhaps moribund because of treatment of gentian violet, in saline, for there was no detectable relationship between the change in size and the length of time between the body temperature observations and room temperature observations. The standard deviation is high (about 90 % of the mean difference), and complete analysis of variance showed that different cells differed demonstrably in their tendency to shrink. This could not be attributed to a tendency, which might be readily expected, for larger cells to shrink more than smaller cells, that is, for the percentage shrinkage to be constant from

cell to cell. Between the decrease in diameter and the diameter at body temperature the coefficient of correlation had the non-significant value of +0·341. Two other possible reasons for the difference in shrinkage between the different cells may be mentioned: (*a*) differences in the constitution of the cells; (*b*) the inaccuracy with which the actual fall in temperature may have been indicated by the thermometer on the haemocytometer. Since the object was simply to determine the existence and average amount of shrinkage, these possibilities were not explored, nor was any attempt made to study the rate of shrinkage during the fall of temperature.

The size changes between room temperature and low temperature were unexpected. In nineteen out of the twenty-six lymphocytes the recorded mean diameter at the lower temperature was smaller, but in seven cells the reverse was true. If the true tendency, such as would be discovered by making more and more such observations, were for decrease and increase (real or apparent) to occur with equal frequency (a one-to-one ratio), samples of twenty-six with discrepancies as great as or greater than in the actual sample would occur by chance in less than 3 % of trials. (Such estimates can be obtained directly from tables such as those of Warwick (1932).) There is therefore a significant majority of cells showing a decrease, and this in itself is sufficient to show that the differences cannot be accounted for by variable (accidental) errors of measurement, which would give an equal ratio of increases and decreases. The mean decrease is 0·583 micrometer unit (about 0·14 μ), which is little more than once its standard error ($\pm$0·405 unit), and therefore, if the series of differences could be represented by a normal curve, the mean would be pronounced non-significant, in apparent contradiction to the fact that there is a significant majority of cells showing a decrease. Further analysis showed the reason for the apparent disagreement. The differences cannot be adequately represented by a normal curve, in which differences between individuals are due only to numerous small factors. There was a difference between the response of the different cells much greater than could be accounted for by observational error. The conclusion is that, while the majority of the cells decreased in size, the apparent increase in seven of the cells was a real increase in at least some of them. The explanation is obscure. It will be recalled that pure water, cooled below 4° C., expands, but it is not clear how the phenomenon or the critical temperature may be different in the complex suspensions and solutions of proteins, salts and other substances that constitute a lymphocyte. So far as the thermometer readings indicated actual cell temperatures, there was no indication that the cells that expanded had been cooled more than those that contracted; nor was there an appreciable relationship between rapidity of cooling and the different response of different cells. Dr E. G. Young, Professor of Biochemistry, at Dalhousie University, with whom the problem was discussed, suggested that the interpretation should probably be sought, not in the physical phenomena of size changes in solutions and emulsions, but rather in a change in osmotic relationships as cells died, a change due to

alteration in the permeability of the cell membrane or in the internal structure of the cell. Where swelling occurred it might be attributed not to change in temperature but to the effect of the light used for illumination in the microscope, because after cells had been exposed to such light Earle (1928) found increase in their size—part of the process of degeneration. This could not, however, account for the shrinkage which occurred in most of the cells, and it should be noted even with reference to the cells that enlarged, that the exposure to light was short—only the 3 or 4 min. occupied by each observation.

No further attempt has yet been made to solve the problem, because the immediate object was secured—the demonstration that in the study of cell size the temperature at which measurements are made, or at which the cells are treated with fixatives, is important. It might be thought that frozen section technique might eliminate size changes due to chemical fixation, dehydration and embedding, but it is now obvious that one cannot assume any simple relationship between cell sizes at body temperature and cell sizes in frozen sections.

Comparison of lymphocyte sizes in saline and in films

From twenty-one different punctures in the fingers of the same person as in the above temperature experiments, drops of blood were taken. The first drop was wiped off, the second was diluted with saline as in the temperature experiments, and from it ten lymphocytes were measured in the haemocytometer at body temperature (with very little variation from 37° C.). The third drop was likewise diluted, for measurements that are not recorded here. From each of the fourth and fifth drops a film was prepared, without saline dilution, by drawing the drop along a slide with the edge of a square cover-glass. The films were stained with Wright's stain and mounted under balsam. Several months after all the measurements in saline had been completed ten lymphocytes were measured in one film from each puncture at room temperature with the same microscope, objective and micrometer and at the same magnification as the cells in saline. The results are shown in Table V.

In all except two out of the twenty-one punctures the haemocytometer mean was greater than the corresponding film mean, and in the two exceptions the discrepancy was so small that it could easily be accounted for by error of measurement. The following quantities were estimated:

Mean of twenty-one differences: 8·107 micrometer units (about 1·9 μ).

Standard deviation estimated from twenty-one differences: $\pm$4·286 units.

Standard error of mean: $\pm$0·935 unit.

The mean difference is 19·3 % of the mean (about 10 μ) of the twenty-one sets of mean diameters of cells in saline; and, since the mean difference is over eight times its standard error it is very highly significant. The "true" difference that would be approached by making more and more observations under these conditions might, however, lie anywhere within twice the standard error of the mean in the present sample, without the actual sample being considered

unusual (significantly different from the true value). On the same basis, in any given experiment involving ten lymphocytes in saline and ten in the corresponding film, the difference cannot be prophesied more closely than twice the standard deviation, that is, from about −1 to +17 micrometer units.

The standard deviations in Table V represent the variation from one lymphocyte to another in the same drop. Analysis showed that there was no appreciable difference between this variation in films and the corresponding variation in saline mounts. Since the mean sizes were less in the films, the variation expressed as a percentage of the corresponding means (the coefficient of variation) was significantly greater in the films than in the saline mounts.

Table V. *Comparison of lymphocyte sizes in saline mounts and films*

(From each puncture ten lymphocytes were measured in saline mounts and ten in films. The sizes are means of the ten observations, in eyepiece micrometer units.)

Puncture no.	Mean diameters of ten lymphocytes		Mean diameter in saline *minus* mean diameter in film	Standard deviations	
	Saline	Film		Saline	Film
1	42·24	31·26	10·98	3·46	4·77
2	42·27	35·11	7·16	3·63	5·50
3	40·40	41·53	−1·13	1·40	3·06
4	41·94	35·82	6·12	3·04	3·30
5	42·62	33·51	9·11	2·03	3·53
6	45·06	46·68	−1·62	4·00	3·13
7	44·99	35·16	9·83	5·13	2·19
8	43·75	29·42	14·33	2·53	2·80
9	43·66	30·30	13·36	3·28	1·69
10	43·21	35·48	7·73	3·34	2·48
11	42·39	31·15	11·24	3·65	4·68
12	41·61	31·42	10·19	1·99	2·65
13	41·26	37·09	4·17	1·53	3·24
14	43·65	29·52	14·13	3·10	1·44
15	40·96	35·01	5·95	2·69	2·60
16	40·98	36·26	4·72	3·13	2·04
17	41·29	30·92	10·37	3·42	2·19
18	40·14	34·44	5·70	3·08	3·77
19	41·64	30·23	11·41	3·35	2·61
20	39·92	31·63	8·29	1·71	2·18
21	40·00	31·80	8·20	2·70	2·83

In the interpretation of the difference in size between lymphocytes in films and those in saline mounts, only a small part can be attributed to fall of temperature from that of the body to that of the room. Several more plausible factors claim attention. First, although undoubtedly the conditions in the saline are more like those of the blood than are the conditions under which the film is prepared, it may be asked: how far might the difference be produced by actual swelling of the lymphocytes, which might arise, even in saline that was isotonic for the red cells, if the lymphocytes had been rendered moribund by the gentian violet used as a stain? For the previously stated reasons no extensive observations with supravital stains were made, but the appearance of the cells, in comparison with supravital preparations, and the fair agreement in lymphocyte size between our preparations and the supra-

vitally stained cells of Wiseman (1931) makes it doubtful that the whole difference could be attributed to swelling of the cells in saline. Moreover, the size variations oppose that suggestion. If the swelling, due to absorption of water, had been so great as to produce these differences, the same process might reasonably be expected to increase the size variation between the cells, because on the average the larger cells would take in more water than the smaller cells. The variation was just as great in the films as in the saline mounts.

It may next be recalled that previous observations (Mainland *et al.* 1936) showed a tendency for the smaller lymphocytes to be near the centre of films, and it may be asked whether the difference between sizes in films and sizes in saline arose because the cells measured in saline were more representative of the whole drop, whereas in the corresponding films only some cells near the centre were measured. Again, if this were the complete explanation, the variation between cells in saline should have been greater than the corresponding variation in the films, and this was not so.

Our results agree with Ponder's (1934) statement regarding red cells in films as compared with those in saline, but they disagree with Wiseman's (1931) demonstration that lymphocytes in films were larger than in the corresponding saline mounts. Although the number of cells measured by Wiseman in each film or saline mount was greater than in our series, his number of independent comparisons between films and saline mounts was smaller than ours. His results show no analysis to allow for chance differences between samples, and various relevant details such as the temperature at which his measurements were made are omitted. The discrepancy between our results and his may be partly due to the difference in his method of preparing and staining films, and it may simply be all due to the irregularity of size distribution of lymphocytes, so that we measured cells where they tended to be small and Wiseman measured them where they tended to be larger. This illustrates the difficulty of interpreting differences when, as is usual, measurement of all the cells in a preparation is impracticable.

It was not the object of the investigation to find exactly why the saline and film sizes differed, but to compare with the film technique a saline technique which, on the whole, should reproduce more closely the conditions of the blood. The comparison involves an examination both of mean sizes and of variations between cells, because both of these are important in any biological study. The general ideas and methods are applicable to any such comparison between, for example, two methods, one of which is more accurate but more difficult than another.

Relationship of mean lymphocyte sizes in saline mounts and films

The lower mean size in films would not necessarily be a disadvantage if there were a close relationship between the mean sizes in films and those in saline mounts. The series of twenty-one pairs of observations (Table V) shows

no evidence of such a correlation (a non-significant coefficient of 0·125). It is likely that the differences between the mean sizes obtained from the twenty-one saline preparations, although significant, were largely due to technique and did not represent changes in the lymphocytes in the blood, because some of them occurred between drops obtained within a few hours of each other. It is possible that comparison of film and saline technique on different people whose lymphocytes actually differed in mean size would show a correlation between the results of the two techniques, but the great variation in the differences shown in Table V indicates that the film technique cannot be a satisfactory method of indicating lymphocyte sizes in the corresponding saline mounts in any individual case.

Variation in mean size in films and in saline mounts

Between the twenty-one pipettes the variation in mean size was much greater in the films than in the saline mounts, the difference being highly significant.

Saline mounts:

Mean of twenty-one items (Table V): 42·094 micrometer units (approx. 10 μ).
Standard deviation estimated from twenty-one items: $\pm$1·526 units (approx. $\pm$0·36 μ).
Coefficient of variation: 3·63 %.

Films:

Mean of twenty-one items (Table V): 33·988 units (approx. 8 μ).
Standard deviation: $\pm$4·100 units (approx. $\pm$0·97 μ).
Coefficient of variation: 12·06 %.

Consistency (low variation) between different measurements does not necessarily show the superiority of a technique. It may indicate a low sensitiveness towards real differences, or it may be due to bias; but in this case the reasonable interpretation is that the film technique itself increased the variation. It should be recalled that variation between the cells in films is no greater than in saline mounts, and, in view of the earlier results in this paper, the great variation between films can perhaps best be interpreted as due to mechanical factors influencing the spread of lymphocytes on slides, and differing in their result from slide to slide. Such added technical variation is very undesirable, because real differences, for example between the blood of different persons, have to be all the greater in order to manifest themselves. The very satisfactory low observational variation between saline mounts should be emphasized.

Variation between cell sizes

It is still not always realized that, in any study of sizes of living things both in pure and applied biology, the variations between individuals are as important as is the mean size. A great variation may indicate the admixture of several strains or groups of individuals, each group with a different mean size, but not sufficiently distinct to produce separate modes (peaks) in the frequency distribution graph. For example, the great size variation in human ovarian connective tissue nuclei (Mainland, 1931) is partly to be explained by the occurrence of two types of cell. Increased variation may also indicate pathological changes. Sterility in horses and bulls is associated with a higher variation in spermatozoon head lengths than is found in fertile animals of the same species (Savage *et al.* 1927, 1930). There is greater variation between nuclear sizes within the same specimen in uterine carcinoma than in non-malignant mucosal hyperplasia (Mainland, 1933).

Lymphocyte sizes vary more from each other than do red cell sizes (Mainland *et al.* 1935) in films taken either from healthy or diseased persons, and if variation is to be used as an indication of pathological differences it is desirable to find out how much of it can be attributed to the technique of film preparation. As shown above, the variation from cell to cell, expressed as standard deviations in Table V, was not appreciably different in saline mounts and films, and the variation expressed as a percentage of the mean was greater in films than in saline mounts. This is not what would be expected if the mean size differences of lymphocytes between films and saline mounts were due to some simple process such as the loss of water by evaporation from the cell or by exosmosis on account of the increased salt concentration of the fluid around the cells during the drying of the film. These processes would, on the average, presumably reduce the differences in size between cells because of the greater amount of water lost by the larger cells; and similarly if the larger size of cells in the saline mounts were due to simple intake of water by endosmosis, the variation between these cells should be greater than in the films. Doubtless the process is highly complicated. Some cells in the drying films may adhere more than others to the serum proteins and therefore not be able to contract as much as others. Some cells will die more quickly than others and their change in structure at death will alter the amount and rate of osmosis and evaporation before they adhere to the serum proteins. Although the staining solution shows no evidence that it can account for the differences between one film and another, it may produce size changes within the first few seconds after it is applied, and these size changes may differ from cell to cell and so contribute to the variation. Whatever may be the causes, the low cell-to-cell variation, relative to the mean, in saline mounts is another point in favour of the saline technique. Although the absolute variation shows no significant difference between films and saline mounts, this does not

indicate that the variation in a film affords any precise indication of what the variation would have been if a saline mount had been used instead.

Because of Earle's (1928) observation that cells, including lymphocytes, swell when exposed to light, the sizes of the lymphocytes in each saline mount were examined to find out how much, if any, of the variation between them might be attributed to the exposure to light during the half hour or so occupied by the measuring. The order in which they had been measured indicated approximately the relative lengths of time during which the cells had been so exposed; the correlation between this and the cell size varied widely among the different mounts, sometimes being negative and sometimes positive. There is therefore no reason to attribute any of the variation to the cause suggested.

THE FREQUENCY DISTRIBUTION OF CELL SIZE

In any series of measurements of cells or objects in biology the position in which individuals of various sizes are placed with reference to the mean is often very important. It will be recalled that the frequency distribution of adult statures of persons of the same sex in a fairly homogeneous population forms a symmetrical figure practically identical with the normal curve of statistics, whereas the corresponding weight distribution is skewed, with individuals farther away from the mean above than below. The diameters of nuclei in fixed and stained sections of human uterine carcinoma and of non-malignant hyperplasia show the same kind of skewness (Mainland, 1933). Skewness of frequency distributions of spermatozoon head lengths is associated with diminished fertility (Savage *et al.* 1927). When cell sizes are increased by experimental poisoning of animals, an increased skewness is often seen (Wermel *et al.* 1932). The increase in size of a few cells can thus be detected, without much increase of the mean size being detectable.

In blood films lymphocyte sizes exhibit skewness of the same general type as do weight measurements of adult human beings. Where no more than a hundred cells in any film had been measured, skewness might be due to the measurements being made chiefly on small centrally placed cells, only a few of the larger more peripherally placed cells being included. This cannot be the sole cause, for the same type of skewness was found when all the lymphocytes in one film were measured (Mainland *et al.* 1936). Full analysis of skewness is elaborate, but a simple test, although less sensitive, is adequate if it gives results that could not be attributed to chance. This method is the comparison of the distance between the mean and the maximum size with the distance between the mean and the minimum size. In seventeen out of the twenty-one saline mounts (Table V) the maximum was farther away from the mean than was the minimum—a significant majority. The skewness therefore does not only occur in films, and, as a considerable part of the area of the saline mount was observed, as compared with the amount observed in the films, it does not appear as if it could be attributed to the way in which the cells are distributed in the saline mounts.

Great caution must be observed in the interpretation of skewness and of differences in skewness. In comparing a symmetrical distribution of linear measurements such as statures with a skewed distribution such as that of the weights of the same persons, it is important to recall the relationship between linear measurements and volumes (to which weights correspond). For simplicity, let it be supposed that the series consists of spherical cells, of which the smallest has a radius of 2 units and the largest a radius of 4 units, while the majority centre around the average of 3 units. The frequency distribution of linear measurements, it is supposed, is symmetrical. The volumes corresponding to the three radii mentioned will be in proportion to the cubes of the radii, that is, a minimum of 8 units of volume, a maximum of 64, and a central group with 27 units. There is a much greater distance from the centre to the maximum than from the centre to the minimum; the distribution is skewed. In view of this, the skewness of distribution of linear measurements of lymphocytes should not be accepted at its face value. Although no figures can yet be shown, it seems reasonable to attribute at least part of the skewness to the flattening of the cells as they lie in saline or films. As can be seen with mercury droplets, when liquid or semi-liquid droplets resting on a surface are flattened under the influence of gravity, the larger droplets lose relatively more height than do the smaller droplets, and therefore linear measurements, such as were made on the lymphocytes, are more closely representative of volume in the larger than in the smaller droplets or cells. Even if there were no such skewness of lymphocyte volume distribution, or of linear measurements when the cells were all spherical, the observed skewness could thus be accounted for by change of shape.

Such matters have always to be considered in interpreting frequency distributions. If, for example, cells from control animals are compared with cells from experimental or diseased animals, and if the cell size is expressed as the mean of the long and short horizontal axes, a change in shape without volume change may readily introduce or alter skewness. For simplicity, height can be neglected. Then, if a cell of circular outline (diameter 4 units) has become elliptical with a major axis of 8 units, then, to give the same area, the minor axis must be 2 units, and the mean of the two axes will be 5 units, instead of 4 as when the outline was circular.

VARIATION IN MEASUREMENT

In measuring cells the unit of the measuring instrument, for example the micrometer, gives no indication of the accidental (chance) error of measurement. Duplicate readings of each cell increase the labour, but they not only increase the accuracy of size determination, but, as illustrated above, they enable one, by analysis of variance, to decide whether observed differences between cells, for instance at different temperatures, can be attributed to variations in measurement or not. If they are to give full indication of this variation, duplicate length and breadth measurements should be completely

independent, that is, after the first length reading the micrometer should be rotated for the first breadth reading, then rotated back for the second length reading, and so on. In many of the sets of measurements in the present investigation the duplicate readings were made chiefly to increase the accuracy, two length readings being made first and then two breadth readings. Therefore the error of measurement estimated from these is presumably to some extent an under-estimate, but not sufficiently so to invalidate any of the conclusions drawn in the above discussions. Since the size of the cell was expressed as the mean of the length and breadth, the problem was to find how far one estimate of such a mean (from one length and one breadth) might differ from another such estimate made on the same cell. When all the duplicate readings that contributed to Table V were used, they were equivalent to a sample of 420 estimates of mean diameter made on one cell, and, apart from the difficulty of making so many observations on one cell, the duplicate readings made allowance for whatever difference in accuracy may have arisen because of the difference in definition of different cell boundaries. It was supposed at first that the accuracy in the saline mounts would be less than in the films, because of the common tendency for fixed and fully stained preparations to have more distinct outlines than are found in liquid mounts, but there was no appreciable difference in the observational error in the two sets of preparations, and therefore the final estimate of this error was made by combining the two.

The standard deviation representing the error of measurement (the variation between the estimations of the mean diameter) was $\pm 0{\cdot}684$ micrometer unit (about $0{\cdot}16\,\mu$). Hence, if a pair of cells is picked at random, or if one cell has been treated in one way and another in another, or if the same cell has been measured before and after treatment (one length measurement and one breadth measurement being made in each case), the estimated mean diameters must differ by more than $2 \times \sqrt{2} \times 0{\cdot}684 = 1{\cdot}934$ units (about $0{\cdot}46\,\mu$), before one can, with the usual convention regarding significant differences, be reasonably sure that the apparent difference is due to anything more than variation in measurement. If on each cell duplicate readings have been made, the estimated mean diameters are significantly different if they differ by $1/\sqrt{2}$ times this, that is, 1·368 units (about $0{\cdot}32\,\mu$).

Errors in measurement are often expressed in percentage form. The value 1·934 units is 4·6 % of the mean of all the lymphocytes measured in saline and recorded in Table V (42·09 units); whereas it is 5·7 % of the corresponding mean for films (33·99 units). This form of expression does not imply that the smaller cells have the larger error; but it is a convenient way of showing the rather high relative value of the average range of error to be expected, that is, the rather low degree of precision in single observations.

It is unnecessary to give details regarding the errors of measurement in each part of the work and for each observer. No result was accepted unless it was beyond the range of these errors and of other observational variation. In any part of the work where measurements had to be compared with each other, the same observer made all the measurements.

DIFFERENCES IN LYMPHOCYTES BETWEEN DIFFERENT GROUPS OF FILMS

Three different methods of preparing films have been used in this investigation, and the mean lymphocyte diameters are:

Control series (p. 380): 9·4 μ.

Films in Table III (p. 383): 9·2 μ.

Films in Table V (p. 390): 8·0 μ.

The first and third items were obtained from the same person's blood at an interval of perhaps a year, the second item from the blood of another person. To find the causes of the real or apparent differences would be difficult, and the chief point to note is that comparisons of sizes, for example between different persons, are legitimate only when the method of preparation and observation is constant. Inaccurate knowledge of magnification can itself introduce serious discrepancies.

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SUMMARY

Cell size studies are important in pure and pathological cytology if fallacies of all kinds are more rigorously excluded than is customary.

Blood from two healthy men was used, films being stained by Wright's stain, and isotonic saline mounts being tinted by gentian violet. Sizes (means of length and breadth) were determined by micro-projection or eyepiece micrometer.

The great lymphocyte size differences in twenty-nine films from the same person (90–100 lymphocytes per film) could not be attributed to controllable differences in rate of drying before or after staining, or in time occupied by staining; nor to differences in punctures made on different days, or order of emergence of drops from punctures. Important fallacies were illustrated by cells measured in blood mixed, before drying, with isotonic saline, hypotonic saline, and isotonic formaldehyde; for example, the possibility of apparent abnormality of cell size in exudates being due to omission of cells too distorted to measure.

In 15 drops of blood spread between cover-glasses the lymphocyte diameter on the lower cover-glass was on the average 0·56 μ greater than on the upper. In spite of size changes during preparation, this was best attributed to more rapid falling of larger lymphocytes, and, along with a previous study of cell size distribution in films, showed that physical factors such as gravity probably produce important size differences where cells are measured in films or droplets.

Twenty-six lymphocytes in isotonic saline were on the average about $\frac{1}{2}$ μ

smaller at room temperature (24–27° C.) than at body temperature. Some shrank farther in passing to a lower temperature (4–13° C.); others enlarged. This suggests the fallacy of cell measurement in frozen sections.

From each of twenty-one finger punctures in one man 1 drop of blood was diluted with isotonic saline and another spread as a film. The mean diameters (ten lymphocytes per film or saline mount) were: films, about 8 μ; saline mounts, about 10 μ. Films gave no reliable indication of sizes in corresponding saline mounts. Size variation between lymphocytes within films was relatively greater than within saline mounts, and variation between films was much greater than between saline mounts. Saline mounts give a much better opportunity of detecting physiological, pathological or experimental differences.

Skewness in frequency distributions of cell sizes is discussed.

Errors of measurement were studied by duplicate readings.

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LYMPHATICS OF THE SKIN, WITH A NOTE ON LYMPHATIC WATERSHED AREAS

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INTRODUCTION

The lymphatics of the skin have been described by various workers since Sappey gave his account of them in 1874. Sappey described the lymphatics of the skin as a network of vessels that occupied the superficial third of the dermis with tributaries that descended to the plexus from the skin papillae. He maintained that no vessels were to be found in the deeper layers of the dermis, but that trunks draining the network in the superficial layer passed vertically downwards through the deeper layer to join the subcutaneous vessels. He vigorously denied the presence of a plexus at the junction of the true skin and the subcutaneous tissue. Bartels (1909) and Von Brunn (1897) contented themselves with a reproduction of Sappey's diagram and a résumé of his description.

Sampson Handley (1922) does not believe that there are any plexuses in the skin. The skin, he holds, is divided into small lymphatic areas $\frac{1}{3}$–$\frac{1}{2}$ in. in diameter, each drained by an independent vertical trunk into the plexus on the deep fascia. This vertical trunk receives as its tributaries papillary lymphatics, small vessels superficially in the dermis and larger ones more deeply placed. These vessels, according to Sampson Handley, do not form plexuses at all; the plexiform appearance found at the junction of the superficial and middle thirds of the dermis, and in the subcutaneous tissue is due, according to him, to these levels being planes of confluence. Sampson Handley lays great stress on the fact that each area of skin draining into a vertical subcutaneous trunk is an isolated unit with little or no communication with neighbouring areas.

Jossifow (1930) describes two capillary nets. First a superficial plexus lying in the papillary layer of the skin, and second a deep net of broad capillaries lying in the subcutaneous connective tissue with trunks descending to the deep fascial plexus.

The descriptions of Rouvière (1932) and Hudack & McMaster (1933) are similar to that of Jossifow, while Yamao (1932) described a sparse network occupying the whole thickness of the corium of the palm of the hand.

Truffi (1934) showed that, on direct surface examination of the skin, two lymphatic nets can be seen with oblique communicating branches between them. Both these plexuses lie in the dermis, and the deeper one communicates with the subcutaneous vessels. None of the dermal vessels, he states, show

the presence of valves. Thus there appears to be two main views on the lymphatics of the skin:

(1) That the dermis is occupied by from 1 to 3 plexuses which anastomose freely.

(2) That there is a separate independent cluster of lymph vessels for each portion of skin of from $\frac{1}{8}$ to $\frac{1}{2}$ in. in diameter, each lymphatic unit having very little or no communication with adjoining units except by way of the deep fascial plexus.

This investigation was undertaken to determine if possible which of these views is anatomically accurate, and also to establish what degree of communication exists between two catchment areas on either side of a lymphatic watershed.

TECHNIQUE

Full-time foetal skin was used for the most part, but several injections were also made on amputated adult limbs. The results of injection of the adult material were unsatisfactory. The foetus was in most cases allowed to putrefy in a warm place for from 14 to 21 days in order to allow the lymphatics to become distended with the gases of putrefaction, and so facilitate the flow of the dye. However, one of the best specimens obtained was one that had been injected when the foetus was in fresh condition.

When the work was begun in Glasgow University, Prof. D. M. Blair kindly placed at my disposal a quantity of foetal skin from various unspecified regions of the body that had been injected by himself some years previously. These specimens, and those prepared by myself, were injected with Gerota's mass using the technique described by Jamieson & Dobson (1910). Intradermal injections were made and, while investigating the watershed areas, the needle was inserted at from 2 to 3 cm. from the watershed line, which was marked after excision of the skin by notching either end of the specimen.

When examining these specimens at a later date a fine copper wire was inserted into the notch at either end and stretched over the skin surface, thus clearly marking the watershed zone.

Skin was taken from the following areas of the body: palm of hand, sole of foot, arm, forearm, leg, thigh, abdominal wall and thoracic walls.

Several attempts were also made to perform a retrograde injection of the cutaneous and subcutaneous lymphatics of a limb. This was done by dissecting out the neurovascular bundle in the axilla, in the groin, or behind the medial maleolus. The bundle was ligatured proximally and the needle inserted into the sheath surrounding the vessels and nerves. By moving the needle about inside the sheath it was hoped to rupture some of the lymphatic trunks and so facilitate entrance of the dye. Two methods of injecting the dye were tried. Injection under gravity was found to be unsuitable owing to the low specific gravity of the injection mass, so the use of a syringe was found to be necessary. On the whole this method of retrograde injection was found to be unsatisfactory, very few successful results being obtained.

After injection the specimens were fixed in 10 % formalin and dehydrated. Some were cleared at once in benzol followed by benzol-benzoate, and examined uncut. Thereafter sections 1 mm. thick were cut by hand, using a safety-razor blade, magnifying spectacles being worn. The cutting of thick sections enables one to follow the continuity of the lymphatic vessels more readily, and to trace the capillary lymphatics to the main trunks at a deeper level. This was also the experience of Ssysganow (1930). Other pieces of the skin were treated by the celloidin method. All sections were mounted by the Spalteholz method (J. T. Wilson, 1924).

DESCRIPTION OF MATERIAL

On surface examination of cleared uncut material from the skin of the sole of the foot and of the anterior thoracic wall, vast plexiform arrangements of injected vessels are seen. In the specimen from the sole of the foot the vessels

Fig. 1. Surface view of skin of sole of foot (× 21) showing vessels of superficial dermal plexus. Vessels of the deeper dermal plexuses can also be seen just out of focus.

can be followed over an area of more than 1 in. in diameter without any absolute break in continuity in any of the dermal lymphatic plexuses (Fig. 1). There is no suggestion of small localized areas, each with an independent series of lymphatic vessels, completely or partially isolated from neighbouring areas. Four sets of vessels lying at different levels in the skin and subcutaneous tissue can be seen.

By focusing down through any particular area, each plexus can be brought into focus in turn. The most superficial plexus consists of thin-walled vessels of fairly uniform calibre. These show no valvular dilatations but, where a

vessel branches or two or more join, there is a dilatation which presents an angular appearance (Fig. 2). These superficial vessels outline polygonal areas, and into the vessels of this superficial dermal plexus smaller twigs pass from the areas included in the meshes of the net. These initial twigs end in tapering extremities, and it seems probable that still smaller ramifications may arise from these which are too small to be demonstrated successfully by injection. These superficial vessels can be seen to arise from a deeper set which forms the

Figs. 2, 3, 4. Skin of sole of foot (× 110). These figures show four sets of vessels in the same area of skin lying at varying depths from the surface. These photographs were taken without moving the specimen. Fig. 2 shows ramifications of the superficial dermal plexus. Fig. 3 demonstrates the intermediate dermal plexus the vessels of which form the network of polygonal areas. The deep dermal plexus is here shown out of focus. Fig. 4 shows the vessels of the deep dermal plexus and a subcutaneous trunk.

intermediate dermal plexus. This plexus is composed of vessels which show greater variations in calibre than do the superficial set (Fig. 3). Some appear to be of approximately the same diameter as the vessels of the superficial plexus, while others are from four to six times as broad as those in the upper net. This plexus, like the superficial one, shows angular dilatations where vessels meet, but there are no valves present, thus depriving this layer of the moniliform appearance so characteristic of lymphatics. The vessels outline polygonal areas. These vessels of the second plexus can be followed down to a third network of vessels, the deep dermal plexus. These vary greatly in calibre; some are of the same calibre as the vessels in the intermediate dermal plexus, while others are as much as six times as wide as the more superficial vessels (Fig. 4). Another feature of the vessels of this third plexus is that they possess

Fig. 3.

Fig. 4.

valves, and that very sudden changes in calibre may be found, a relatively large vessel ending in two vessels of greatly reduced diameter. These vessels outline irregular areas like the more superficial ones, smaller branches filling up to some extent the interstices of the net formed by the larger trunks. By focusing still deeper, occasional very large lymph trunks may be seen lying at a relatively great depth from the surface.

The appearances presented in sections vary greatly, and at first sight do not seem to correspond to those seen on surface examination of the uncut material. This, however, can be explained by the varying calibres of the vessels of the intermediate and the deep dermal plexuses, the superficial plexus appearing in some cases to be predominant and to pass directly into the deep dermal plexus, while in other cases all four types of vessels described above can be seen clearly, the fourth set being of course subcutaneous and not dermal.

The most striking feature is the extremely free anastomosis which exists throughout the entire thickness of the dermis (Fig. 6). No vessels were demonstrated in any part of the epidermis which, in many of the specimens, was well preserved. In other cases desquammation of the epidermis to a greater or less degree had occurred, and in some specimens, owing to the difficulty in clearing the stratum corneum, the epidermis was scraped off to obtain satisfactory surface views of the dermis.

The vessels of the superficial dermal plexus occupy the superficial third of the thickness of the dermis. They begin as minute channels occupying the centre of each dermal papilla, run vertically and pass into the vessels forming a free network at the base of the dermal papillae. The vessels of the superficial network freely anastomose, are of fairly uniform calibre and possess no valves (Fig. 6). In the skin of the anterior thoracic wall, as distinct from the other regions examined, the superficial dermal vessels form a deeper anastomosing network. Here there are two or sometimes three nets of minute vessels superimposed on one another from the superficial layers of the dermis inwards, instead of a single network with vertically projecting branches as seen in other regions. The spaces between any two sets of horizontally running vessels are bridged by vertical or oblique channels, and so a very complete anastomosis is found in the superficial layer of the dermis.

The vessels of the intermediate plexus typically occupy the middle third of the dermis, but very often they appear to extend down to the transition of dermis to subcutaneous tissue. These vessels are of varying diameter and possess no valves. The vessels of the superficial plexus flow into those of the intermediate plexus and, in most cases, the transition from one to the other is gradual (Fig. 5). The deep layers of the dermis and the superficial zone of the subcutaneous tissue is the site of a third plexus—the deep dermal plexus. Here again the calibre of the vessels varies greatly. In typical sections there is a gradual increase in the size of the vessels from those in the dermal papillae down to the subcutaneous ones, but in some sections of the skin of the sole of

the foot the change between the intermediate and deep plexus is extremely abrupt. In that case the intermediate plexus ends in very large dilated trunks which may run horizontally and form a true plexiform arrangement, or may pass vertically downwards through the subcutaneous tissue lying in the fascial strands separating the fat lobules, anastomosing with one another and eventually terminating in the deep fascial plexus. These vessels of the deep dermal plexus, which are partly dermal and partly subcutaneous, possess typical valves and have a beaded appearance.

Fig. 5. Section of skin of sole of foot (×36). Superficial dermal plexus, *A*; intermediate derma plexus, *B*; deep dermal plexus, *C*; subcutaneous trunk traversing subcutaneous tissue obliquely and arising by confluence of deep dermal vessels, *D*.

The lymphatic plexuses in the skin of the sole of the foot and the palm of the hand are much more dense than in other regions of the body. However, the vessels in the skin of the palm of the hand are smaller in calibre than those of the sole of the foot. On occasion the vessels of the deep dermal plexus may occupy the whole of the lower half of the dermis, and appear as a dense network of large vessels with obvious valvular dilatations.

In all three plexuses vessels may run for relatively long distances parallel to the surface of the skin and to one another, with oblique channels connecting vessels lying at varying levels.

SUBCUTANEOUS LYMPHATIC TRUNKS

From the vessels of the deep dermal plexus the subcutaneous trunks commence. These vessels are larger than the vessels of the deep dermal plexus and have valvular dilatations. They do not vary greatly in calibre from their commencement in the deep dermal plexus to their termination in the deep fascial plexus. They arise usually singly at regular intervals from the deep

dermal plexus, but may sometimes arise in pairs and are usually formed by the confluence of several deep dermal vessels. After passing inwards for a short distance, two or more of these vessels may unite to form a single larger trunk, or each vessel may pursue an independent course through the subcutaneous tissue. They tend to run in fibrous septa separating the lobules of fat in the subcutaneous tissue, and their course is more often oblique than perpendicular. Sometimes their course is so oblique that the vessel forms a very acute angle with the surface of the skin, and in some cases may appear almost parallel to the skin surface. The very oblique vessels pursue a more sinuous course than those which are more nearly at right angles to the skin surface. Very often the subcutaneous vessels exhibit arcade formation. These lymph-vascular arcades connect the vessels that are running obliquely towards the deep fascial plexus. The transverse vessels forming the arcades are curved with their convexity towards the surface and may lie near the junction of dermis and subcutaneous tissue. The deep fascial plexus receives tributaries at more frequent intervals than those of $\frac{1}{3}$–$\frac{1}{2}$ in. demanded by Sampson Handley's theory.

This description differs from all those previously published, with the exception of that of Yamao, who states that the dermis contains two or three capillary lymphatic nets depending on the area investigated. In the axilla he found two or three and only one in the palm of the hand. Sappey describes only one net and that lying in the superficial third of the dermis.

Jossifow describes two plexuses, one in the superficial layer of the dermis and the other in the subcutaneous tissue. This deeper plexus seen by Jossifow may correspond to the deep dermal plexus which sometimes traverses the dermis and sometimes lies in the subcutaneous tissue.

According to Truffi there are two networks of lymphatics in the dermis communicating with the subcutaneous vessels, but he denies the presence of valves in any dermal vessels. Their presence has, however, been definitely demonstrated in the deep dermal plexus, and this agrees with the findings of McCallum.

The lymphatic pattern of the skin is found to be much the same in all areas, except that in some (e.g. sole of foot) there is a richer plexus than in other areas. One striking feature is the very free anastomosis between the vessels of all three plexuses as seen both in sectional and surface view. In sections the longest stretch of continuous plexus seen was $\frac{1}{4}$ in. in extent; but, on surface view, vessels could be followed in the skin of the sole of the foot over an area 1 in. in diameter without any appreciable break in the continuity of the plexus.

From the above descriptions it appears that, in all the areas investigated, there are three plexuses of vessels in the dermis. The two superficial ones possess no valves, while the one most deeply placed shows well-marked valvular constrictions. These vessels are all thin-walled, but this is particularly true of the superficial net. The vessels of all three plexuses are arranged so as to outline polygonal areas, and the interstices of the network are occupied by smaller

ramifying vessels that drain into the larger surrounding trunks. From the deep dermal plexus larger subcutaneous vessels run obliquely through the subcutaneous tissue to the deep fascial plexus, and the vascular arcades unite these obliquely running subcutaneous vessels.

Also on several occasions two or three vessels have been seen passing into the deep fascial plexus within a distance of 0·76 cm. It seems most unlikely, therefore, that there is only a single subcutaneous trunk draining each area of the skin $\frac{1}{3}$–$\frac{1}{2}$ in. in diameter. If two such vessels are seen in one section it follows that other vessels of a similar nature may pass down to the deep fascial plexus from the same area on one or other side of those displayed in the section. Further, if each area of skin has a self-contained lymphatic drainage, evidences of this should be demonstrable in the form of breaks in the plexuses when an extensive area showing injected vessels is examined from the surface. Even though actual breaks in the plexus are not seen, zones showing anastomosis of smaller vessels would be expected. Neither of these features has been observed.

LYMPHATIC WATERSHEDS

Sappey and Sampson Handley have described the lymphatic drainage of the skin and its appendages as being subdivided into six areas by two horizontal planes through the umbilicus and clavicles, and by a third plane vertically through the mid-line of the body. The lymph from the skin and subcutaneous tissue of each of these areas drains into a particular set of lymph glands, and the zones or watersheds separating these catchment areas are occupied by small tortuous vessels forming an anastomosis between the vessels of two adjacent areas. According to Handley, however, these zones are never traversed by trunk lymphatics.

Injections were made 2–3 cm. on either side of a watershed zone, with a view to determining the extent of the anastomosis at these areas, and also whether the separation between the catchment areas is functional or anatomical. All specimens were examined in surface view before being sectioned. Injections were made above and below a horizontal line through the umbilicus, on both sides of the mid-line of the anterior abdominal wall, and on both sides of the mid-line of the anterior and posterior thoracic walls.

Injection of the deep dermal and subcutaneous vessels was much more successful than of the superficial and intermediate plexuses. This may have been fortuitous, or may mean that at the watershed areas the dermal vessels are smaller and more sparse than elsewhere. However, where the injection of the dermal vessels has been successful, they form three plexuses as they do in other areas.

It has also been found that where the dermal vessels in the watershed zone have not taken the injection, neither have the dermal vessels in an indifferent area of skin on the side of the injection area away from the watershed. Therefore there are certainly dermal vessels occupying the watershed zone, and these vessels are similar to those in areas of skin distant from the watershed.

In surface view the uncut material vessels of the intermediate and deep dermal plexuses are clearly seen, as also are subcutaneous trunks. These vessels cross the watershed and unite injection areas on either side of that line. Those of the deep dermal plexus and the subcutaneous vessels are very obvious, the trunks crossing freely from one side to the other. The vessels can be seen crossing the watershed at right angles (Fig. 6); they may cross it obliquely or may cross the zone and then run parallel to it.

On one occasion a large vessel was seen to cross the watershed from the injection area on one side to near the injection area of the opposite side, and

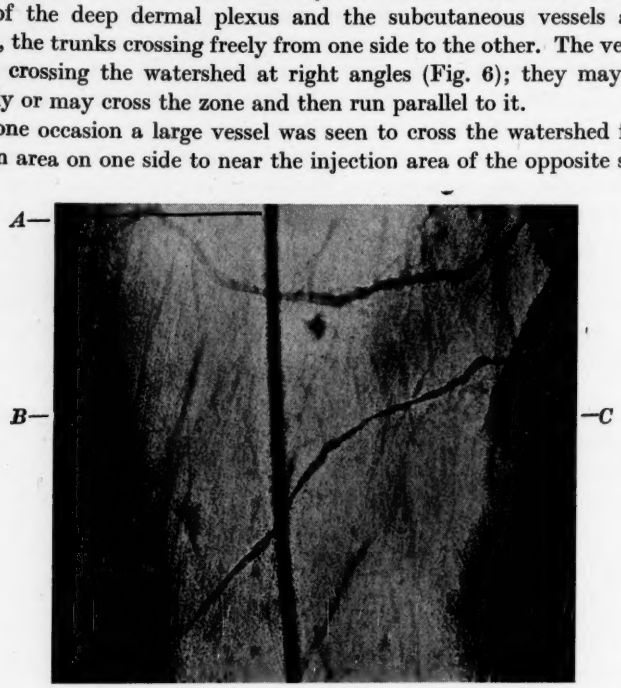

Fig. 6. Surface view of skin of anterior abdominal wall at the watershed zone on a horizonta plane through the umbilicus (× 12·5). Mid-line of watershed zone marked by wire, *A*; injection areas, *B* and *C*. Two lymph trunks uniting injection areas and crossing watershed area.

then revert to that side from which it originally started. As an alternative to a direct unbroken course over the watershed zone, these subcutaneous vessels and the vessels of the deep dermal plexus may branch to form a very dense network of vessels in the watershed area or they may outline more or less quadrilateral areas as have been described above in regions distant from any watershed. The vessels of the deep dermal plexus and the subcutaneous ones possess valves. The valves were carefully examined to ascertain whether there was any change in the direction of the valves after the vessel had crossed the watershed.

In some specimens one can demonstrate conclusively that the valves on the two sides of the watershed open in opposite directions (Fig. 7). In other cases, however, while valves can be seen, the cusps have either been obliterated by masses of dye or so distorted that it is impossible to state in which direction they open.

This change in direction of the valves makes a very probable explanation of the watershed phenomenon. Vessels of the intermediate dermal plexus can also be found in the zones between catchment areas. All sections were cut at right angles to the watershed line. In them vessels of all three plexuses can be seen occupying the thickness of the dermis in the watershed area. The vessels in this area are as numerous as in an indifferent area of skin distant from the watershed, and those of the deep dermal plexus are partly dermal and partly subcutaneous. The presence of numerous subcutaneous trunks crossing from one area to another is confirmed. In addition to dermal and subcutaneous vessels those of the deep fascial plexus also cross the watershed zones.

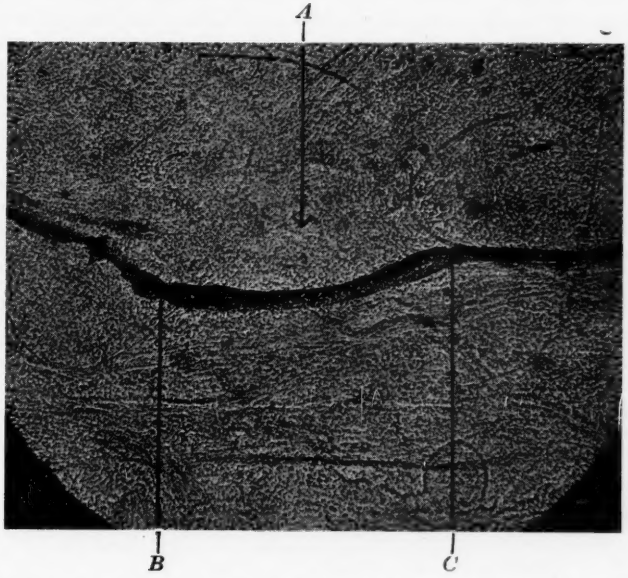

Fig. 7. Section of skin from anterior aspect of thoracic wall including mid-line watershed zone (×32). This shows a subcutaneous trunk crossing the watershed zone and uniting two injection areas. Mid-line, *A*; valves, *B* and *C* opening in opposite directions.

It appears therefore that the degree of communication between two catchment areas at the watershed zone is very much freer than has been supposed. As this state of affairs can be demonstrated by injection of lymphatics only where the valves have been rendered incompetent by putrefaction, probably there is a physiological watershed at the areas indicated. However, in certain pathological conditions (e.g. carcinoma of the breast) lymph flow may occur in either direction.

This functional watershed will not involve the superficial and intermediate dermal vessels where valves are absent, but the deep dermal vessels and the

subcutaneous ones possess valves which open in opposite directions. Thus lymph which passes from the dermis to the neutral area, between two opposing valves on either side of the watershed zone, may flow in either direction, while that which enters beyond the first valve on either side can flow in one direction only.

SUMMARY

1. A superficial, intermediate and deep dermal plexus of lymphatics is present in the skin. The deep dermal plexus communicates with the deep fascial plexus by subcutaneous trunks running in fibrous strands separating the lobules of subcutaneous fat. Very free anastomoses of dermal lymphatics and no evidence of independent drainage from localized areas of the skin has been found.

2. Description of the presence of the communication of the dermal, subcutaneous, and deep fascial vessels over the lymphatic watersheds. Valves in deep dermal and subcutaneous vessels found to open in opposite directions on either side of watershed.

I wish to express my thanks to Prof. D. M. Blair of Glasgow University for placing his material at my disposal, for granting facilities for the work in his Department, and for his interest, encouragement, and helpful criticism throughout. I am also indebted to Prof. Low for the provision of facilities enabling me to continue the work in his Department.

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THE CERVICAL VERTEBRAE OF THE AUSTRALIAN NATIVE

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Two definite facts have been recorded concerning the condition of the cervical vertebrae of the Australian native. Broad noted in 1902 that they are "small, but well formed": and Owen, in 1851, and Cunningham, in 1886, remarked on the small size of the individual vertebrae and the usual non-bifid condition of the cervical neural spines. Both these characteristics are very typical of the average cervical vertebrae of the aboriginal. All the cervical vertebrae of the native are very lightly built when comparison is made with the condition characteristic of European bones: and it is well known that one of the physical distinctions of the native is the apparent shortness of his neck. But it is not perhaps the absolute smallness of the whole cervical series that is the most striking feature: for it is in the relative diminution in certain members of the series, and in certain definite elements, that the distinction lies. Fortunately, the measurements of a wide series of European vertebrae are available. The more recent work of Cyriax has supplemented the earlier records of Anderson and Dwight and we may have assurance in making statements concerning the average size of European bones.

According to the findings of these workers the average transverse diameter of the atlas is 78·7 mm., varying from 63 to 90 mm. The average of my series of aboriginal bones is 72·5 mm., varying from 68 to 80 mm. The average antero-posterior diameter of the European atlas is 45·41 mm., varying from 39 to 53 mm. The same measurements of the aboriginal bone show an average of 42·2 mm. with a variation from 38 to 47 mm. The average aboriginal atlas is therefore some 6 mm. shorter between the tips of the transverse processes and 3 mm. shorter in the antero-posterior diameter than the European bone. It is also built upon finer and more slender lines; the posterior arch, in particular, being as a rule a very narrow bar of bone.

It is the axis, however, that shows the greatest and most constant differences from the condition typical of the European. Macalister described and figured (*op. cit.* Pl. X, fig. 20) an axis vertebra in which the lateral processes are almost completely hidden under the superior articular surfaces. This specimen is an extreme example of his cryptotrematic type in which, when viewed directly from above, both arterial foramina are hidden from view. Macalister does not give the provenance of this particular bone; but he mentions the fact that, for the purposes of his paper, he had examined the bones of

Australians. The figure so perfectly depicts the condition typically present in the Australian (compare his Fig. 20 with the present Fig. 2) that one is tempted to suspect that the bone illustrated is actually that of an Australian native. Macalister gives the extreme intertransverse width of this particular specimen as 45 mm. For the European axis, Dwight gives the average intertransverse

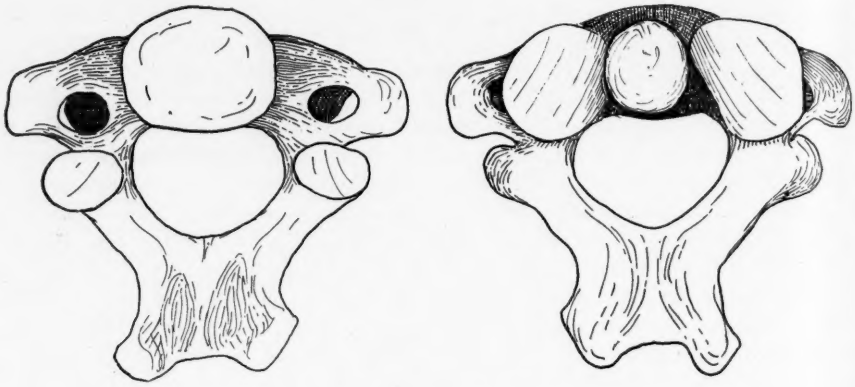

Fig. 1. The axis vertebra of a European, lower and upper surfaces, natural size. Diopterographic tracing.

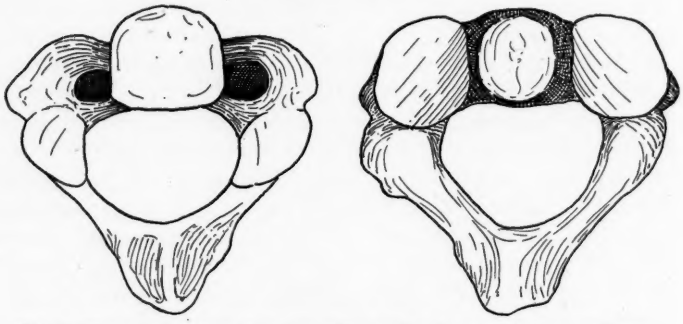

Fig. 2. The axis vertebra of an Australian native, lower and upper surfaces, natural size. Diopterographic tracing.

width as 55 and Cyriax as 54·49 mm. The average of the same measurement in a series of aboriginal bones is, however, 45·2, a very great reduction of the size typical of the European. The antero-posterior diameter of the axis in Europeans is 50·59 mm., while in the Australian it is only 41·7 mm. and it may be as small as 37 mm.

It is therefore evident that, though the aboriginal atlas is smaller in its two diameters than the European bone: this smallness is disproportionately displayed in the axis. For the aboriginal atlas falls short of European standards in

these two diameters by 9·41 mm., whereas the axis is reduced by 18·19 mm. Now this marked reduction in the size of the bone is due almost entirely to the small size of the transverse processes and the well-known diminution of the neural spine. For it is a strange fact that the transverse diameter of the spinal

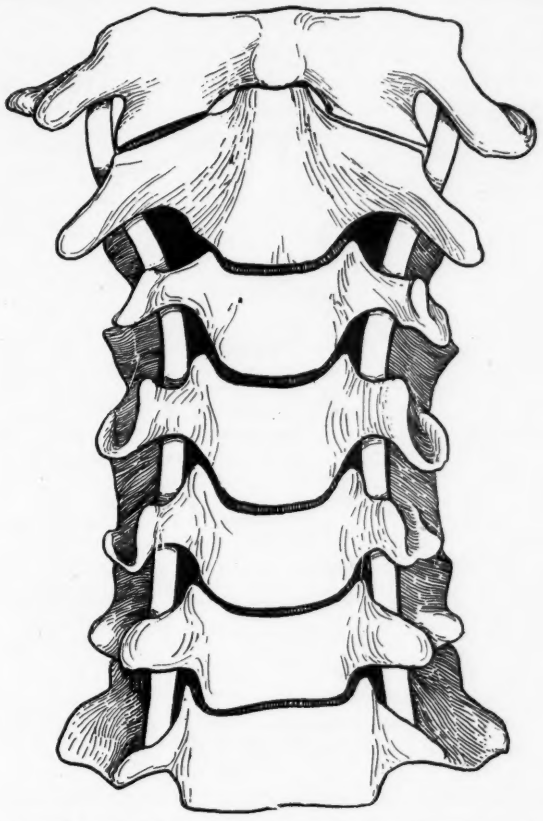

Fig. 3. The cervical vertebrae of a European, natural size. Diopterographic tracing.

canal of the aboriginal axis is absolutely almost as large as, and relatively far larger than, that of the European. Macalister gives the average breadth of the neural canal as 22·5 in European bones, having an intertransverse diameter averaging 55: while in the Australian the internal measurement is, on the average, 22·2 in bones with an intertransverse diameter of 45·2. Moreover, whereas the spinal canal at this level is, as Macalister states, cordiform in the European, it is nearly circular in the Australian.

It will be noticed by contrasting Figs. 3 and 4 that the cervical portion of the column of the Australian is considerably shorter than is that of the

European. (The average height of the adult Australian is 167·7 for males and 156·2 for females.) Apart from its shortness, its contours show a striking difference from those of the European, since the relative width of the first and seventh members of the series accentuate the relative narrowness of the second and, to a lesser degree, of the intermediate elements. But the most marked

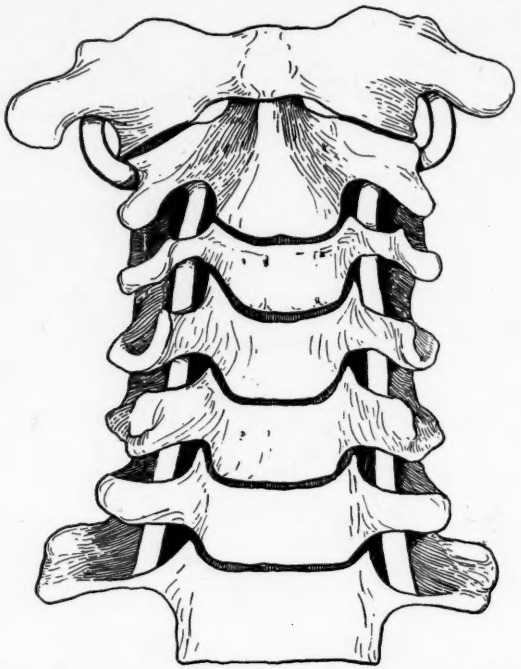

Fig. 4. The cervical vertebrae of an Australian native, natural size. Diopterographic tracing.

distinction is concerned with the foramina for the vertebral artery as it passes through the axis vertebra. Seen from below, the typical site of entry of the canals in the European is situated some little distance from the lateral margin of the body (see Fig. 1): in the Australian the foramina are placed immediately alongside the body (see Fig. 2). Since the whole breadth of the axis of the Australian is so much less than that of the European this means that, at this level, the vertebral arteries of the two sides are much more nearly approximated. In many cases the separation of the foramina at the lower border of the body of the axis is only half the distance typical of the European. The approximation of the lower ends of the canals causes them to be situated immediately below the median aspects of the superior articular facets. As the canals pass upwards they, therefore, take a right-angled bend and emerge by laterally

directed orifices to the immediate lateral aspects of these facets. The standard description that "the costo-transverse foramen is directed very obliquely upward and laterally" could never be applied to the condition present in the Australian axis; for here the costo-transverse canal consists of two parts, a vertical below and a horizontal above, meeting each other at right angles beneath the superior articular facet. The course of the vertebral artery in the Australian native differs somewhat from that typical of the European, for whereas in the latter it passes upwards through the series of costo-transverse foramina with a more or less gentle curve, in the Australian the two arteries approach each other steadily until they reach the lower border of the axis and then turn abruptly outwards to reach the foramen in the atlas. It would be interesting to ascertain if this type of cervical vertebrae, so characteristic of the Australian, is present in like degree in any other races.

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THE COURSE OF THE INCISOR BRANCH OF THE INFERIOR DENTAL NERVE IN RODENTS AND SOME OBSERVATIONS ON THE NERVE SUPPLY OF THE PULP

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During the course of another investigation, it became apparent that the normally accepted view of the course of the inferior dental nerve in the rodent did not altogether correspond with the actual conditions which exist in this order of mammals, and for this reason we considered that the subject would merit a further investigation.

One of the features by which the rodents are distinguished from the other orders of mammals is the presence of large incisor teeth of continuous growth, a feature which is also found in the grinding teeth of certain species. It is assumed that these immense incisors have been evolved from a much smaller ancestral tooth which was entirely situated in the anterior part of the jaw. In the course of time, the tooth was gradually lengthened and pushed the apex farther and farther posteriorly in the jaw. Such an evolution of the teeth is used to explain a peculiar course of the inferior dental nerve as it is described in the textbooks. Such an account will be found in the 2nd edition of Tomes' *Dental Anatomy* and reads as follows:

> **The large scalpriform or chisel-like incisors extend far back into the jaw....The length and curvature of these incisors relieve from direct pressure their growing pulps, which come to be situated far back in the jaw. The open end of the lower incisor, for example, being in many species actually behind the last of the molar teeth. The nerve going to supply the persistent pulps is of very large size, and, owing to the open end of the tooth having formerly occupied a more anterior position in the jaw, runs forward beneath the tooth and then bends abruptly backwards to reach the tooth-pulp.**

This account has been repeated in all the subsequent editions of the book and a similar description will be found in other manuals. We have illustrated in Fig. 1 *a* this peculiar recurrent course of the nerve which is apparently caused by the backward projection of the apex of the tooth.

In the present investigation, the course of the nerve was studied in the rabbit and afterwards in the rat. In the former animal an attempt was first made to carry out the dissection by chipping away the bone and opening up the inferior dental canal. This method, however, proved to be unsatisfactory on account of the hardness of the material. To overcome this, the mandibles were partially decalcified in dilute nitric acid until the bone became sufficiently

softened to permit of its being cut with a scalpel. This made dissection much easier, but care had to be taken in the region of the teeth which were, of course, also decalcified. When the nerve was displayed in this manner, it was found that after it entered the bone at the mandibular foramen, it first of all ran downwards and forwards and then horizontally forwards along the lower border of the middle portion of the mandible. In this part of its course, the nerve lies in close proximity to the bases of the grinding teeth. Just in front of these teeth, the nerve divides into a large mental and smaller incisor branch. The former, passing towards the outer side of the jaw, runs obliquely forwards

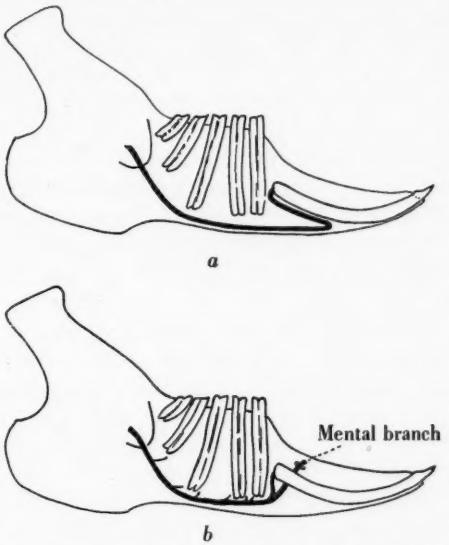

Fig. 1. *a*, Recurrent course of incisor nerve as usually described; *b*, Actual course of incisor nerve as seen in the rabbit.

and upwards to emerge from the bone at the mental foramen. The smaller incisor branch turns vertically upwards and enters the posterior end of the incisor tooth. From the main trunk of the inferior dental nerve, fine twigs arose which could be traced upwards to supply the molar and milk-molar teeth. These are the fibres which form the plexus around the bases of the teeth as described by Olivier (1928) and by Starkie & Stewart (1930). The diagram (Fig. 1*b*) illustrates the course of the nerve as has been described above. This was found to vary but little in the various specimens which were examined. From the above description it will be seen that in the rabbit the peculiar looped recurrent course which is given in the textbooks does not occur. As in the rabbit, however, the length of the incisor tooth is relatively short, further dissections were made in the rat, where the incisor is very much longer and

passes backwards beneath the molars almost to the posterior extremity of the jaw. It was felt that if such a recurrent nerve were to be found, it ought to be present in the rat. The jaws were again decalcified and the nerve dissected out as before. In the rat, the mandibular foramen is placed just behind the posterior end of the incisor, and the inferior dental nerve after entering the foramen ran medially to this extremity of the tooth. Here it divided into its two terminal divisions some distance behind the 3rd molar tooth. The larger external mental branch ran forwards with a curved course in relation to the lateral aspect of the incisor. It took no part in the innervation of the tooth and finally emerged at the mental foramen a little in front of the 1st molar. The smaller internal branch ran forwards and downwards between the roots of the molar teeth and the concave upper surface of the incisor. It broke up into numerous branches to supply the teeth. The twigs which ran upwards supplied the molars and the remaining branches, which were of larger size, passed downwards to reach the upper border of the incisor tooth. It was not possible with the naked eye to determine exactly how these branches to the incisor tooth ended, and it therefore became necessary to prepare histological sections to settle this point. Lower jaws of the mice were stained by the Cajal silver technique, embedded in paraffin and cut in serial sections in the antero-posterior plane. In this manner it was possible to trace the finest bundles of fibres throughout their course. The mouse was chosen for this purpose on account of the size of its jaw and observations show that the arrangement of the nerve was similar to that which was seen by the naked eye in the rat.

A naked-eye examination of the mandibles of the rat did not reveal any branches from the inferior dental nerve passing to the pulp of the incisor teeth, but this could be clearly demonstrated in the histological preparations of the jaw of the mouse. In these specimens it was observed that the apical opening into the pulp chamber of the incisor was slit-like in shape and was situated on the upper aspect of the tooth near its posterior extremity. Under the microscope, branches of the inferior dental nerve could be traced into the pulp through this opening. One particular nerve bundle was of special interest. It arose from the main branch after it had passed beyond the opening and in order to gain the pulp, the bundle had to bend sharply backwards to reach the opening in the tooth. This appears to be the only nerve with a recurrent course and it is completely different from the type of recurrent nerve described in textbooks. In the first place, it was a very small bundle and it was not the sole nerve supply to the pulp of the teeth. Moreover, the length of its recurrent course was not more than 1 mm. and finally, it lay in relation to the upper surface of the tooth. The other nerve fibres which we have described as passing downwards towards the upper surface of the tooth appear from our histological preparations to be going solely to the periodontal membrane. In Fig. 2 the course and distribution of the inferior dental nerve in the rat, as described above, is shown in a diagrammatic manner, and the recurrent branch to the pulp of the incisor as seen in a mouse has also been added to give a complete

picture of the arrangement of the nerve. We have thus been able to show that in three of the commonest of the rodents there appears to be no evidence for the existence of a large recurrent nerve on the under aspect of the incisor teeth. It is of course possible that such a nerve may exist in other species, for this has been described by Owen (1840–5) in the porcupine, but granting that it does exist in this species and possibly in others, it is quite clear that the presence of a recurrent nerve to the incisor teeth cannot be regarded as a general feature in all members of the order Rodentia.

During the course of this investigation, the opportunity was taken of examining histologically the innervation of the pulps of the teeth of these animals. Some years ago, one of us (Stewart, 1927) was unable to find medullated nerves in the pulps of the teeth of continuous growth. This seemed a favourable

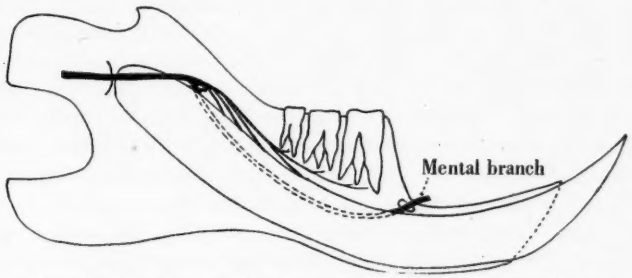

Fig. 2. Course and distribution of the inferior dental nerve in the mandible of the rat.

opportunity to check this observation and also to see whether, if medullated nerve fibres were absent, there was also a lack of non-medullated fibres. This became of even more significance as a paper by Ikada appeared at this time in which he gave an account of nerve fibres in the pulps of molars of rabbits.

Our investigations have shown that Ikada was right and Stewart was mistaken in his earlier view, as we were able to demonstrate the presence of these medullated nerves in numerous specimens. The material used in this part of the investigation consisted of pulps which had been shelled out from the incisor teeth of rabbits, guinea-pigs and rats. These pulps were stained by the Cajal silver technique and embedded in paraffin. Serial sections were then made both in the longitudinal and in the transverse directions. In the rabbit, where the nerves of supply are easily visible, it was possible to remove the pulp with a portion of the nerve attached. A few molar teeth were also treated in the same manner. An examination of the incisors of the rabbit revealed that the pulps of these teeth were very freely supplied with nerve fibres. These ran towards the crown in a direction parallel to the long axis of the tooth. They differed, however, in their relations from that seen in the normal mammal in that the fibres were isolated or consisted of very small bundles containing only two or three fibres. In those pulps in which the nerve bundles from the

inferior dental nerve had been preserved, a curious feature was seen. This large nerve trunk remained intact until it was practically in contact with the pulp, then at first glance it seemed to disappear suddenly. Examination under a higher power, however, showed that this apparent disappearance was due to a rapid division of the nerve into a number of small branches at the base of the pulp, which, after a very short course, broke up into still smaller branches which were very difficult to follow, since subdivision of the bundle was continued until there were no more than three or occasionally four fibres in a fasciculus. This rapid subdivision of the main trunk into fine fasciculi appears to be characteristic of the rodent and differs from that of the ordinary mammal where the number of fibres in the fasciculus is much more numerous. The arrangement of the nerve fibres in the incisors of the guinea-pig and also in the molars of the rabbit was similar to the condition just described. In the incisors of the rat, however, the nerve fibres were much less numerous, and in some pulps there was difficulty in finding examples of axon cylinders. This falls into line with the observations made in the earlier part of this paper, that while the nerves going to the pulp of the rabbit could easily be detected by the naked eye, those in the rat were so small that they could not be detected without the assistance of the microscope. Similar series of serial sections were made from material stained with osmic acid to demonstrate the medullated fibres. It was hoped by this means to determine more precisely the way in which the nerve trunk broke up on entering the pulp, for, as has been mentioned, it was extremely difficult to follow this in the Cajal stained sections. Examination of these sections showed exactly the same arrangement as that already described. There was the same rapid subdivision into bundles of about five to eight fibres, followed by a further rapid subdivision into fasciculi of two or three bundles and into isolated fibres. The myelinated fibres could be traced throughout the length of the pulp and a comparative count showed, as far as could be estimated, that the number of myelinated fibres in the osmic acid sections corresponded closely to the number of axon cylinders in the Cajal sections and demonstrated that the nerve fibres did not lose their myelin sheath on their entrance into the pulp.

SUMMARY

In the specimens of rodents' jaws which have been examined, the nerve to the inferior incisor tooth does not pass forward underneath that tooth and afterwards loop backwards to enter the pulp cavity as is described in text-books. In the rabbit, the nerve having arrived at the apex of the tooth, passes sharply upwards and enters without a recurrent course. In the rat, the nerve lies on the upper aspect of the tooth and the fibres which enter, although some have a short recurrent course, are too small to be seen with the naked eye. In histological preparations, the main nerve bundles to the pulp break up very rapidly either into individual fibres or into small fasciculi of only two or three fibres, this being markedly different from the arrangement in the pulps

of other representative mammals. These nerve fibres are practically all myelinated.

ACKNOWLEDGEMENTS

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ABNORMAL DEVELOPMENT OF THE BRAIN IN AN 8 MM. PIG EMBRYO

By J. S. BAXTER and J. D. BOYD

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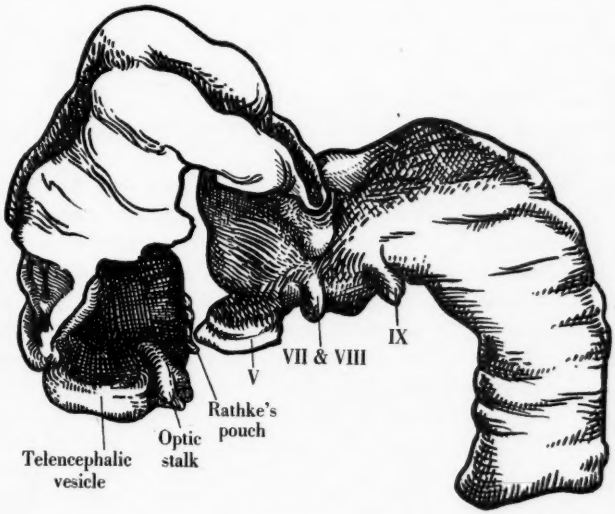
The specimen, which is described here, is one of a series of 8 mm. pig embryos prepared for routine class teaching in this Department. In order to determine its developmental age it has been compared with a number of normal pig embryos from our collection, and it corresponds essentially with a normal pig embryo of 8 mm. C.R. length. We have therefore used an embryo of this size as a standard of comparison in our study of the abnormal embryo. At the outset it should be stressed that the latter is in excellent histological condition. As may be seen from Pl. I, fig. 6, the tissues have been preserved in a very good state, mitotic figures being numerous and clear. It is not an unreasonable assumption then, that this embryo has been obtained and fixed while still in a living condition. In a survey of the literature upon developmental defects in the cephalic part of the central nervous system we have been impressed by the fact that, so far as we have been able to ascertain, all recorded specimens of comparable abnormalities are in a poor histological state; this may, in some cases, be due to post-mortem change; in others, secondary mechanical and pathological processes have obscured the picture of the primary defect. Our specimen, then, is of considerable significance, as its age and histological condition rule out such complications.

DESCRIPTION OF SPECIMEN

The embryo was one of a batch which had been stained in bulk with haematoxylin and eosin, the amnion in each case being intact. It was therefore only after sectioning that the abnormality was discovered. Consequently two reconstructions of the head end of the embryo have been prepared, each made at a magnification of 50 diameters. The first is a plaster of Paris model of the external form of the head (constructed by Lewis's modification of Born's method), while the second is a conventional Born wax-plate model of the corresponding portion of the central nervous system.

Text-figs. 1 and 2 depict the left lateral aspect of these reconstructions. On comparison of the two it is clearly to be seen that a certain part of the cephalic portion of the neural tube has been splayed out upon the surface of the head of the embryo. This eversion of the neural folds extends from the rostral limit of the metencephalon to the neighbourhood of the upper border

Text-fig. 1. External form of the head end of the embryo.



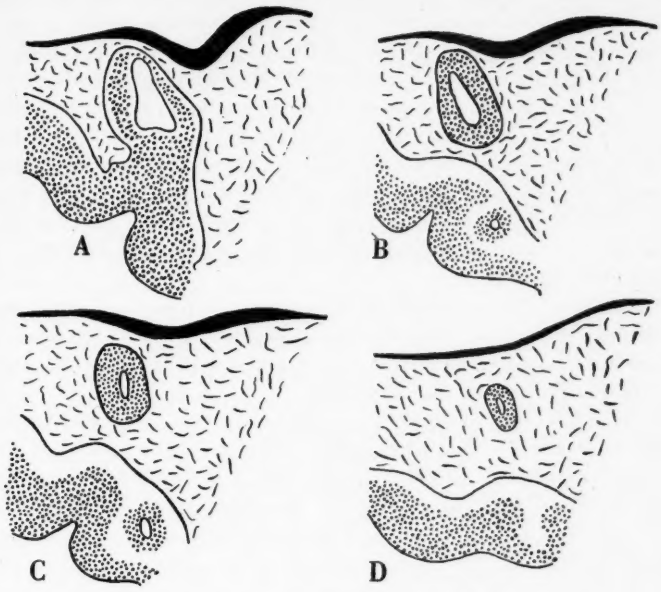
Text-fig. 2. Reconstruction of the cranial end of the nervous system.

of the lamina terminalis. The line of demarcation between the neural tissues and the somatic ectoderm is everywhere quite distinct (Pl. I, fig. 1). The roof of the hind brain appears to be essentially normal in its configuration. The deep cavity to be seen (Text-fig. 2) just rostral to the metencephalon leads caudally into the cavity of the hind brain. The telencephalic vesicles are present on either side, and are comparable in their form and structure with those of the control embryo. Just behind the telencephalic vesicles are the optic stalks, the development of which has proceeded to a rather greater degree on the right than on the left, but even here the condition of the optic diencephalic outgrowth is much arrested as compared with the normal embryo of this stage. In association with this condition there is failure of development of the lens placode. On the left side there is no trace of an ectodermal thickening to form the primordium of the lens; on the right side, where the neural optic evagination is somewhat better developed, there is a slight ectodermal thickening (Pl. I, fig. 4). In the case of the 8 mm. control embryo the lens placode has already separated on each side from the ectoderm and is lying in the primitive optic cup. There has obviously, then, been a marked retardation in the development of the optic primordia in this specimen. In order to lay further stress upon this point we present in Text-fig. 3 camera lucida drawings of four consecutive sections through the optic primordia on the right side of this embryo; and it will readily be seen in these figures that the lens primordium is merely a slightly thickened area of ectoderm in continuity with the general somatic ectoderm. There is only a small depression to indicate its future possible invagination. On the left side neither ectodermal thickening nor indication of invagination are present.

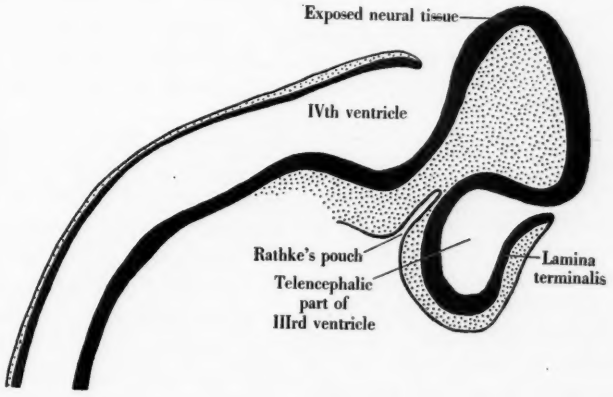
The neural and stomodaeal components of the hypophysis appear to be normal (Pl. I, fig. 5).

The attachments of the trigeminal, acoustico-facial and glossopharyngeal nerves to the brain stem are indicated in the second reconstruction (see also Pl. I, fig. 2). These nerves in their entire course are quite comparable with those of the control embryo. The otic vesicle also appears quite normal. The vagus, accessory and hypoglossal nerves (which however have not been modelled) are normal, but, in striking contrast, there is no trace of the oculomotor, trochlear or abducens nerves. These latter three nerves were clearly to be seen in our control embryo.

Anteriorly the reconstructions present several significant features. There are two foramina of Munro to be seen at the anterior edge of the exposed neural tissue, and they lead into the telencephalic vesicles. Immediately inferior to this level the end of the neural tube is closed so that a small rostral cul-de-sac is formed. We take the closing membrane to be (Pl. I, fig. 3, Text-fig. 4) the lamina terminalis, and the cul-de-sac must then be the telencephalic part of the third ventricle. This is in accordance with the description of Streeter (1912) regarding the two component parts of the third ventricle. Thalamic swellings can be seen exposed on the surface immediately behind the



Text-fig. 3. Successive sections through the right optic primordium.



Text-fig. 4. Sagittal graphic reconstruction of the head end of the embryo.

foramina of Munro. These swellings are separated by a deep cleft which is continued throughout the exposed mid-brain as a median fissure.

A sagittal graphic reconstruction of the brain (Text-fig. 4) emphasizes several of the points previously mentioned. The extent of defective union of the neural folds is shown; the intact lamina terminalis is seen bounding the telencephalic part of the third ventricle and behind this cavity is seen Rathke's pouch. A further point, not hitherto stressed, is the marked upward "buckling" of the whole of the neural plate in the region of the mid brain. This may also be seen in Text-fig. 2. If this embryo had developed further, it is possible that solution of continuity of the nervous system might have occurred in this region (cp. Frazer, 1921).

We have been able to satisfy ourselves that the blood vessels of the head of the embryo are normal. The internal carotid arteries may be seen on Pl. I, fig. 1, and these vessels could be traced throughout their course. The significance of this observation will be discussed later.

The remainder of the specimen calls for no comment except that its condition is everywhere that of an ordinary 8 mm. pig embryo. This is shown in Text-fig. 1 where the external appearance of the branchial arch region is perfectly normal. Particular attention was paid to the primordia of the ductless glands in the study of the sections in view of the stress which has been laid by some writers upon changes in these glands in similar developmental abnormalities of the nervous system. We found no discernible changes from the normal picture of the endocrine gland primordia in an 8 mm. pig embryo.

DISCUSSION

The obvious lesion in this abnormal embryo is non-closure of the neural folds in the mesencephalic and diencephalic regions. Associated with this abnormality is absence of the oculo-motor, trochlear and abducens nerves of each side and defective development of the optic outgrowths and, particularly, the lens primordia.

A comparable abnormality has been described by Sternberg (1927). He had available for study a human embryo of 5 mm. G.L. in which there were three clefts in the dorsal part of the medullary tube. The first of these he judged, from its relations, to be the still open anterior neuropore. There was a further rhomboidal cleft in the region of the mid-brain separated from the first by a connective tissue bridge, while a third small fissure was present in the upper part of the spinal cord. The gross and microscopic condition of this embryo was admittedly poor. Nevertheless certain important features could be made out. A number of the cranial nerves and their attachments to the brain stem were discernible; they were the trigeminal nerve, the acoustico-facial complex, the glossopharyngeal, vagus, and cranial portion of the accessory nerves. Both optic outgrowths were present, each communicating widely with the cavity of the diencephalon. There was a slight degree of ectodermal thickening indicating the lens primordia, more marked upon the right side,

and a minor degree of cupping here to indicate future invagination of the optic vesicle.

The resemblances between this specimen and the pig embryo we have described are striking. In both, a non-union of the neural folds in the mesencephalic and diencephalic regions is associated with defective development of the optic primordia. Further, in the pig embryo there is failure of development of the IIIrd, IVth and VIth cranial nerves. Sternberg does not refer to these nerves in the description of his abnormal embryo. The presence of marked optic abnormalities in the two embryos prevents them from being regarded as early examples of what is usually designated "anencephaly".

What the subsequent developmental history of these embryos might have been can only be surmised. An abnormal human embryo of 35 mm. C.R. length described by Hunter (1934) as a case of "extroversion of the cerebral hemispheres" probably represents such a later stage in so far as the brain itself is concerned. His specimen showed a marked exposure of neural tissue on the surface of the head in the mid- and forebrain regions. This neural tissue was continuous laterally with the body ectoderm but bulged over it. A portion of the exposed tissue showed the structure of normal choroid plexus and projected from the surface into the amniotic cavity. It is clear that the condition of the brain found by Hunter could easily have been derived from an earlier abnormality such as is present in Sternberg's and our specimens. Continued growth of exposed neural tissue in such embryos could easily result in the bulging of this tissue over the somatic ectoderm to produce the condition which Hunter has called "extroversion".

To none of these three specimens can the term anencephaly properly be applied as, in each of them, there is present an intact neural plate, abnormally developed in a certain region, but with no evidence whatsoever of rupture of the brain stem. The latter has been emphasized by Frazer (1921), and later by Mann (1937) as the essential lesion in anencephaly. Thus Mann writes (1937, p. 57): "in any case the essential lesion is a transverse solution of continuity of the brain stem. This will account for most of the known facts and is borne out by examination of a human embryo with the defect in process of developing". The embryo referred to here is the one described by Frazer but, in our opinion, the condition of "anencephaly" is already well established in his specimen and there is no clue to the nature of the primary defect. Frazer and Mann both stress rupture of the internal carotid arteries distal to the origin of the ophthalmic arteries as an important secondary factor in the production of the final condition. They both admit, however, that the primary factor is a cerebral defect due, according to Mann, to "a sudden fortuitous accident occurring during an otherwise normal development rather than a dysgenic process present and acting from the first". This explanation may be applicable to some cases of anencephaly but, if our specimen had continued to develop, a cerebral condition closely resembling anencephaly would probably have resulted. This condition would primarily have been due to

failure of union of the neural folds and any secondary processes resulting in solution of continuity of the brain stem (such as excessive "buckling" of the neural plate or stretching of the internal carotid arteries) are superimposed upon this primary lesion. We find this explanation more acceptable than one which implies normal closure of the neural tube and then invokes supposititious foetal hydrocephalus to rupture the roof plate and initiate the abnormality. Our explanation has the further advantage of bringing anencephalic conditions into line with spina bifida, the former being, in the first instance, examples of encephalon bifidum. The two conditions, spina bifida and encephalon bifidum, occasionally coexist (see Wrete, 1924) in which case there has been non-closure of the whole length of the neural folds.

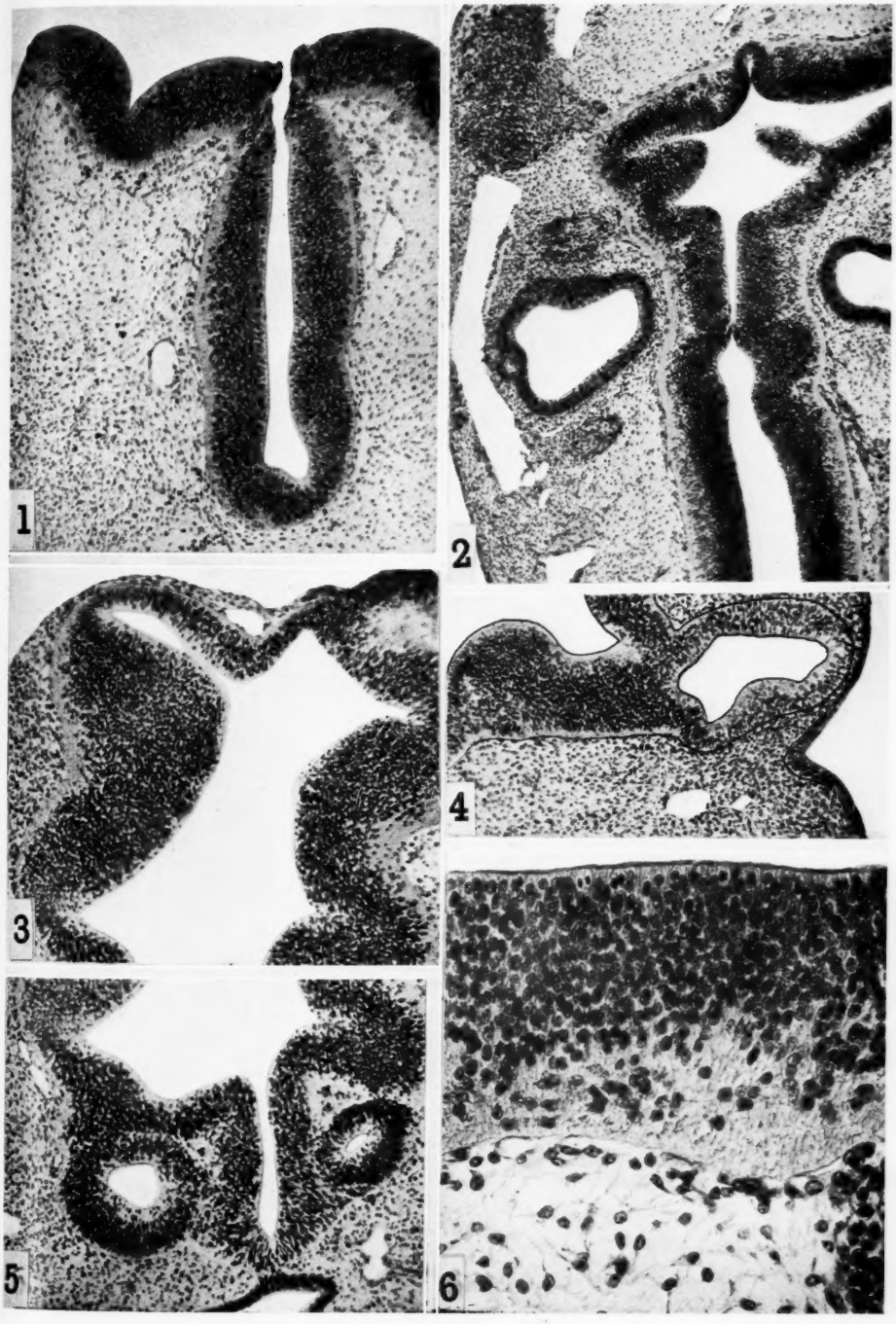
Our conception of the condition known as anencephaly, then, is that it is the result of secondary changes masking the primary lesion which we believe to be non-closure of the neural folds. This may, occasionally, be a fortuitous accident of development, but that it can be a dysgenic, hereditary defect is shown by the fact that in certain inbred strains of rabbits a high proportion of the offspring have anencephaly (Dr J. Hammond, personal communication). The secondary changes which result in frank anencephaly may, in part, be due to the unrestrained growth of the exposed neural tissue and to its upward buckling. To these mechanical effects of non-closure of the neural plate may be added vascular changes (as suggested by Frazer, but which are not present in our specimen), interference with normal nervous metabolism as a result of the disorganized production and circulation of the cerebrospinal fluid and the exposure of the nervous tissue itself on the surface of the embryo.

It is impossible to be dogmatic about the relation between the non-closure of the neural folds in the mesencephalic and diencephalic regions of our embryo and the abnormal development of its optic apparatus and the associated nerves. We incline to the opinion that the two are closely connected and that the lesion is essentially a dysgenic one involving those parts of the embryo which have to do with the development of the visual apparatus.

Our thanks are due to Miss Joan Loewenthal for her kind assistance in the preparation of Text-figs. 1 and 2.

SUMMARY

An abnormally developed nervous system in an 8 mm. pig embryo is described. The abnormality consisted in non-closure of the neural folds in its mesencephalic and diencephalic regions, the complete absence of any trace of the IIIrd, IVth and VIth cranial nerves and markedly retarded development of the optic primordia. The excellent histological condition of the sectioned material enables one to state that no secondary pathological changes had supervened. The relationship of this abnormality to anencephaly is discussed.



BAXTER AND BOYD—ABNORMAL DEVELOPMENT OF THE BRAIN IN PIG EMBRYO.

UNIV. OF MICH.

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EXPLANATION OF PLATE I

Fig. 1. Section through the diencephalic region of the embryo. The exposed neural tissue is shown and, on the left side, its continuity with the somatic ectoderm can be seen. Note the intact internal carotid arteries on either side of the basal portion of the nervous system. (×85.)

Fig. 2. Section through the hind brain. On the left side can be seen the trigeminal ganglion with its sensory root, the acoustico-facial ganglion, the ganglion superius of the glossopharyngeal nerve and the vagus nerve. The otocysts are seen on either side of the hind brain. (×55.)

Fig. 3. Section to show the lamina terminalis region. (×85.)

Fig. 4. Section through the right optic outgrowth. The thickening of the ectoderm where the lens should have developed is shown. (×85.)

Fig. 5. Section through the optic stalks near their attachment to the diencephalon. The upper part of Rathke's pouch is seen in the lower part of the microphotograph making contact with the floor of the IIIrd ventricle. (×85.)

Fig. 6. Section at a higher magnification through a portion of the exposed neural plate. The excellence of the histological condition of the sectioned material is obvious. Mitotic figures can be seen on the surface of the exposed medullary plate epithelium. (×250.)

THE MORPHOLOGY AND PERMEABILITY OF THE ROOF OF THE FOURTH VENTRICLE IN SOME MAMMALIAN EMBRYOS

By HENRY COHEN and SARAH DAVIES

From the Department of Medicine, University of Liverpool

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I. INTRODUCTION

In a previous communication (Cohen & Davies, 1937) it was reported that in the chick embryo the first escape of cerebrospinal fluid from the central nervous system was coincident with the development of the choroid plexuses of the brain. Two membranous areas, one anterior and the other posterior, in the roof of the fourth ventricle functioned as the sites of fluid escape. The objects of the present investigation are: (1) To discover whether or not a similar correlation exists in mammalian embryos. (2) To identify specialized membranous areas, if such exist, in the roof of the fourth ventricle of these types. (3) To ascertain whether such areas allow of any fluid escape during embryonic life and, if so, whether their permeability is in any way influenced by injections of a hypertonic solution into the blood stream.

II. SURVEY OF LITERATURE

Very few workers have investigated the morphology of the roof of the fourth ventricle in vertebrates during development.

In 1917 Weed recorded the presence of two membranous areas, one anterior and the other posterior, in the roof of the fourth ventricle of pig and of human

embryos at different stages of development. The anterior membranous area is a transitory structure disappearing early in embryonic life. The posterior area, on the other hand, is persistent and forms the greater part of the inferior choroid velum. He also identified an anterior membranous area in the embryos of chick, rabbit, sheep and cat.

Further, Weed replaced the fluid within the central nervous system of living pig embryos at various stages of development by a 1 % double solution of potassium ferrocyanide and iron ammonium citrate. Subsequent fixation in an acid medium secured precipitation of Prussian blue (ferric ferro-cyanide) *in situ* and thus revealed the path taken by the injected solution. Weed found that the injected fluid escaped into the surrounding mesenchyme through the membranous areas in the roof of the fourth ventricle, the first escape being coincident with the development of the choroid plexuses of the brain.

Keegan (1917), investigating rabbit and chick embryos, recorded the presence of an anterior and posterior membranous area in the roof of the fourth ventricle. A detailed description of the complete history of these areas is not given. He injected a 1% double solution of potassium ferrocyanide and iron ammonium citrate into the central nervous system of living embryos of various ages, but found that no extra-ventricular spread occurred, even at stages when the choroid plexuses were well developed.

According to Flexner (1929), a membranous area is present in the roof of the fourth ventricle of amblystoma embryos. In the earlier stages of development it forms the major portion of this roof and is continuous throughout, showing no differentiation into anterior and posterior portions. The subsequent disappearance of this area during later stages is brought about by metamorphosis of its constituent cells into typical ependymal cells and by the development of the choroid plexus.

Owing to the size of amblystoma embryos, injections were not made into the central nervous system. Flexner assumes, however, that the membranous area in the roof of the fourth ventricle functions as the site of extra-ventricular passage of fluid. The basis of his assumption is that a corresponding area in mammalian embryos has definitely been shown (Weed, 1917) to perform this particular function.

Cohen & Davies (1937), investigating chick embryos, identified and described two membranous areas in the roof of the fourth ventricle, one anterior and the other posterior to the developing choroid plexus. The anterior area is a transitory structure, disappearing at the 9th day of incubation, while the posterior area persists beyond this stage. Injections of a 0·8 % double solution of potassium ferrocyanide and iron ammonium citrate were made into the central nervous system of living embryos of different ages. It was found that an extra-ventricular spread of the injected solution occurred, first through the anterior area and later through the posterior area. The first escape of fluid was correlated with the development of the choroid plexuses of the brain.

Much work has been done to determine the influence of varying salt

concentrations in the blood on the cerebrospinal fluid circulation in adult Mammalia. Of particular interest are the observations recorded by Nañagas (1921), Weed (1922), Foley (1923), and Forbes *et al.* (1928).

Nañagas (1921) replaced the intra-ventricular fluid of both normal and hydrocephalic kittens by a 1 % double solution of potassium ferrocyanide and iron ammonium citrate. This procedure was followed by intravenous injections of a hypertonic salt solution. Subsequent fixation in an acid medium secured precipitation of Prussian blue (ferric ferrocyanide) *in situ* and thus indicated the channels along which the replacement solution had passed. In both normal and hydrocephalic animals it was found that absorption occurred through the ventricular ependyma into the capillary bed of the nervous system. Intravenous administration of a hypertonic salt solution served to hasten the process of absorption. In all animals, normal and hydrocephalic, the epithelial cells of the choroid villi showed no invasion by the Prussian blue granules.

Weed (1922), investigating cats and dogs, increased the salt content of the blood by intravenous injections of a hypertonic saline solution. He then injected a 1 % double solution of potassium ferrocyanide and iron ammonium citrate into the subarachnoid spaces of these animals. His findings are in close agreement with those of Nañagas. The Prussian blue precipitate had passed through the ependymal lining of the cerebral ventricles into the underlying capillary network. No absorption was observed through the epithelium of the choroid plexuses.

Foley (1923) made an investigation on the lines essentially similar to those adopted by Weed. He found that increased osmotic value of the blood caused a retrograde flow of fluid from the subarachnoid spaces into the ventricles of the brain. The distribution of Prussian blue granules indicated that the foreign solution had passed not only through the ependyma but also through the epithelium of the choroid villi.

Forbes *et al.* (1928), investigating cats, injected a 1 % double solution of potassium ferrocyanide and iron ammonium citrate into the subarachnoid spaces. After a short interval, a hypertonic salt solution was administered either intravenously or into the peritoneal cavity. In all cases examined, precipitated Prussian blue granules were observed in the lumen of the vessels in the choroid plexuses.

Thus, according to Nañagas (1921), and Weed (1922), the choroid plexuses take no part in the intra-ventricular absorption of the cerebrospinal fluid even when the osmotic pressure of the blood is increased. On the other hand, Foley (1923) and Forbes *et al.* (1928) state that under the influence of intravenous injections of a hypertonic solution the plexual epithelium absorbs fluid from the ventricles.

A search of the literature has failed to reveal any data concerning the influence of hypertonic solutions in the blood stream on the extra-ventricular passage of cerebrospinal fluid during embryonic life.

III. MATERIAL

The findings herein recorded are based on observations of rabbit, rat and guinea-pig embryos.

Only in the case of rabbits was it possible to obtain embryos of accurately determined ages. In the case of rats and guinea-pigs the dates of mating, and therefore the exact ages of the embryos, were not known. A rough indication of their stage of development was obtained, however, by measurement of the embryos along the mid-dorsal line from the crown of the head to the root of the tail. Since there is considerable variation in size, not only among embryos of the same age from different mothers, but even among embryos from the same uterus, such lengths are clearly no satisfactory criterion of age; nevertheless, they do serve as an approximate indication of the stage of development.

IV. METHODS OF INVESTIGATION

In all cases the mother was anaesthetized either with chloroform or ether. The entire uterus with the embryos *in situ* was quickly removed and placed in normal saline solution warmed to a temperature of 37–38° C.

Immediately prior to injection an incision was made in the uterine wall in such a way that the placenta remained intact. The embryo, still attached by its umbilical stalk to the placenta, was exposed by careful removal of the chorion and amnion and kept in the warm saline solution throughout the injection.

The embryos from each animal were divided into two groups, each group containing at least two embryos. In each embryo of one group injections were made firstly into the blood stream, and then after a period of 4–5 min. into the central nervous system. In each embryo of the other group injections were made into the central nervous system alone.

For the purpose of injection into the vascular system, a 2 % saline solution warmed to a temperature of 37–38° C. was used. The embryonic heart was exposed through an incision in the mid-ventral line of the thoracic wall. The injection was made by means of a short glass tube of 3 mm. bore drawn out into a fine capillary point at one end and provided with a small rubber bulb at the other. A small quantity of the saline solution was injected under very slight pressure into the left ventricle of the heart and this procedure repeated until the blood vessels of the head region became so pale as to be hardly distinguishable.

The fluid used for injection into the central nervous system was a double solution of potassium ferrocyanide and iron ammonium citrate. Other workers in this field (Weed, 1917; Keegan, 1917), who have adopted a similar experimental method, used a solution of 1 % concentration, as this is claimed to be isotonic with mammalian blood. Therefore, in order to make our results valid for comparison with theirs, a solution of similar concentration[1] was used

[1] The solution was prepared by dissolving 0·5 g. of each salt in 100 c.c. of solution.

throughout the present investigation. Since a detailed account of the method employed for injecting the embryonic central nervous system has already been communicated (Cohen & Davies, 1937) a brief statement will suffice here. The solution was injected into the caudal end of the central canal of the spinal cord. Any fluid contained in the central nervous system was simultaneously withdrawn from the lateral ventricle of either cerebral hemisphere, thereby eliminating undue increase in intra-cerebral pressure.

As soon as the injection was completed the embryo, still in the warm saline solution and attached to its portion of the uterine wall, was placed in an incubator at 37–38° C. Here it was kept alive for about ½–1½ hr., according to the size of the embryo, to allow of any possible spread of the injected solution. Owing to the transparency of the integument, particularly in the earlier stages of development, the embryonic heart-beat was easily observed and served as the criterion of prolonged circulation.

The embryos were then fixed overnight in a solution containing 10 % formaldehyde and 1 % hydrochloric acid. At the same time the acid in the fixing agent secured precipitation *in situ* of Prussian blue (ferric ferrocyanide) and thus revealed the path taken by the injected solution. After fixation, the embryos were dehydrated in a series of suitably graded alcohol solutions, cleared in xylol and embedded in paraffin wax of 52 or 56° C. M.P. according to season. They were then serially sectioned in the longitudinal plane at a thickness of 4 μ and stained in Ehrlich's or Weigert's haematoxylin and eosin.

V. THE MORPHOLOGY AND PERMEABILITY OF THE ROOF OF THE FOURTH VENTRICLE IN CERTAIN MAMMALIAN EMBRYOS AT DIFFERENT STAGES OF DEVELOPMENT

(*a*) *Results of injections of a true solution into the central nervous system*

Histological observations are reported below on rabbit, rat and guinea-pig embryos in which the central nervous system was injected with a 1 % double solution of potassium ferrocyanide and iron ammonium citrate.

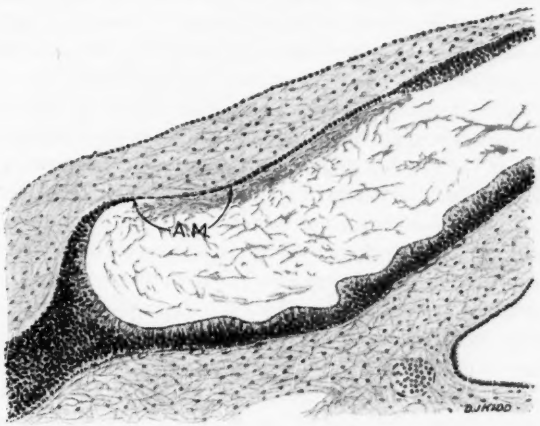
Rabbits.

The earliest embryos which could be injected with any measure of success were those of 11 days. Thereafter specimens were injected at intervals of approximately 24 hr. until the 18 days' stage was reached.

11 *days' embryos.* The wall of the brain stem was wholly ependymal in nature and was of variable thickness. The roof of both third and fourth ventricles was continuous and unfolded throughout and showed no indication of a choroid formation. The roof of the third ventricle consisted of an ependymal layer 3–4 cells deep. The individual cells were columnar in shape and possessed pale granular cytoplasm and round or slightly elongated nuclei. The nucleoli and nuclear membrane were intensely stained in contradistinction to the pale nucleoplasm. The roof of the fourth ventricle was triangular in shape, the wide

base being directed anteriorly and the narrow apex posteriorly. The major portion of this roof consisted of a single layer of cuboidal cells with granular cytoplasm and round clearly defined nuclei. These nuclei were so densely stained that their nuclear membrane and chromatin mesh could only be distinguished with great difficulty. In the anterior region of the roof (see Text-fig. 1) a small median oval area was visible where the cells were extremely flat, consisting of a narrow, elongated nucleus with a thin cytoplasmic investment. This area was abruptly demarcated from the remainder of the roof, its flat cells contrasting sharply with the cuboidal cells of the surrounding epithelium.

The injected fluid was seen wholly within the central nervous system, no extra-ventricular spread of dye being evident. The Prussian blue granules,



Text-fig. 1. Rabbit, 11 days.

intermingled with coagulum, extended in uniform distribution along the central canal of the spinal cord and in the cerebral ventricles. They showed no condensation associated with any particular region in the brain stem.

12 *days' embryos.* The wall of the brain stem was thicker than previously, the greater part consisting of ependyma associated with a narrow stratum of overlying nerve tissue. The thalamencephalic roof, however, was thinner than elsewhere and consisted of ependyma only. It was smooth and unfolded and still showed no indication of villus formation. The myelencephalic roof presented the same general appearance as in the preceding specimens, but the membranous area in its anterior region was relatively larger.

The injected solution still lay wholly within the central nervous system but was no longer uniformly distributed in the cerebral ventricles. There was a slight accumulation of blue granules intermingled with coagulum adhering to, and coextensive with, the ventricular surface of the membranous area in the

roof of the fourth ventricle. The individual cells of this area, however, showed no impregnation with the dye.

13 *days' embryos.* The roof of the thalamencephalon was thinner than in preceding stages but still showed no indication of folding. The roof of the fourth ventricle (see Pl. I, fig. 1) was now divided into anterior and posterior regions by a very shallow depression in the transverse median plane. The cells participating in this invagination tended to be more columnar than cuboidal. Apart from a slight difference in shape they resembled very closely the cells of the surrounding epithelium, showing a similar degree of granulation and wealth of nuclear chromatin content. The membranous area in the anterior portion of the roof was relatively larger than previously, occupying practically the entire roof region anterior to the median depression. In the posterior portion a small, median, oval area was distinguishable where the cells were very low cuboidal in stature. Their nuclei, however, were still rounded or only a little elongated. This slightly differentiated posterior area was not sharply delimited from the surrounding epithelium but showed gradual mergence with it.

The injected fluid was still retained within the spaces of the central nervous system, and was again slightly condensed below the membranous area in the anterior region of the myelencephalic roof. No such condensation was evident below the slightly flattened area in the posterior region, but merely a sparse distribution of granules adhering to its ventricular surface.

14 *days' embryos.* At this stage the median transverse invagination in the roof of the fourth ventricle was more emphasized than previously and its cells more markedly columnar. The majority of these cells retained on the whole their granular appearance. A few, however, were seen to contain a small spherical vacuole lying in the cytoplasm and closely associated with the nucleus. The ventro-lateral walls of the median fold now exhibited a slight degree of undulation, but no true choroid villi were evident. The anterior membranous area in the myelencephalic roof was somewhat smaller than in the preceding stage, but was still abruptly demarcated from the surrounding epithelium. The slightly differentiated posterior area, on the other hand, was relatively larger although it still merged imperceptibly into the general roof layer.

As in earlier stages, the injected solution was still confined within the central canal of the spinal cord and ventricular system of the brain. The blue granules showed a similar degree of concentration below the membranous areas of the myelencephalic roof as in the previous specimens.

15 *days' embryos.* The roof of the thalamencephalon was narrower than hitherto, consisting of a thin ependymal layer 1–2 cells deep. This roof was now slightly undulating and formed several shallow, irregular folds invaded by the overlying mesenchyme. The median depression in the myelencephalic roof (see Pl. I, fig. 2) was deeper than previously and the columnar shape of its constituent cells more pronounced. The undulations in the ventro-lateral walls of this fold, first observed in embryos of 14 days, were here more marked, forming short, unbranched, incipient villi. The epithelial cells of the villi

possessed densely granular cytoplasm and round or slightly elongated, well-defined nuclei. In a large number of these, however, the nucleus with its cytoplasmic investment was relegated to the lower (ventricular) portion of the cell, the upper or aventricular portion being occupied by a large spherical vacuole. In a few cells the reverse condition obtained. The membranous area in the anterior region of the fourth ventricle roof was smaller in extent than previously, but its border still showed an abrupt transition from the surrounding epithelium. The posterior area, on the other hand, showed considerable increase in size, exceeding the anterior area both in length and in width. This area was now sharply delimited from the general roof epithelium and its constituent cells were very flat, consisting of a narrow, elongated nucleus with a scanty investment of cytoplasm.

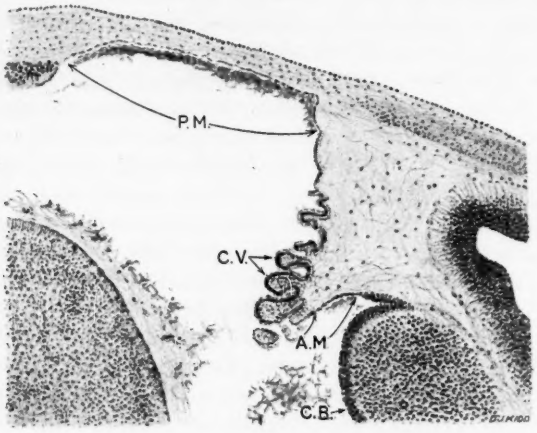
The distribution of Prussian blue granules was similar to that recorded in preceding stages, no extra-ventricular spread of the dye being evident.

16 *days' embryos.* The invaginations of the thalamencephalic roof were more pronounced than previously. Moreover, they now extended anteriorly for a short distance into the lateral ventricles via the foramina of Munro, but did not form true branching choroid villi. At this stage gross morphological changes were evident in the myelencephalic roof. The median transverse fold was extremely well developed reaching almost to the floor of the ventricle. The incipient villi in the ventro-lateral walls of the fold were more marked than hitherto and exhibited a slight degree of branching, forming an embryonic choroid plexus. In section each villus consisted of an axial core of highly vascular mesenchyme surrounded by an epithelium one cell deep. The cells were columnar in shape and possessed pale granular cytoplasm and round, well-defined hyperchromatic nuclei. In the majority of the cells, the nucleus with its investing cytoplasm was relegated to the lower ventricular region of the cell, while the upper or aventricular portion was entirely occupied by a large vacuole. In a few instances the vacuole was so extensive that it practically filled the entire cell, causing the nucleus to be greatly compressed. The anterior region of the fourth ventricle roof was considerably shorter in longitudinal section than previously, owing apparently to encroachment by the developing cerebellar anlage and choroid plexus. Consequently the membranous area of this region was now partly incorporated into the anterior wall of the median transverse fold. This area was still sharply delimited from the surrounding epithelium, but was somewhat smaller in extent than in the preceding stage. The posterior membranous area, on the other hand, was relatively larger, and occupied the greater part of the roof region posterior to the choroid plexus.

The injected dye was still confined within the spaces of the central nervous system. A dense accumulation of blue granules adhered to the ventricular surface of both membranous areas in the medullary roof, but in each case their constituent cells showed no invasion by the dye.

17 *days' embryos.* The degree of undulation in the thalamencephalic roof was much more pronounced than hitherto. The resultant villi extended for a

considerable distance into the lateral ventricles and exhibited a slight degree of branching, forming an incipient choroid plexus (see Pl. I, fig. 3). Each villus in section consisted of a central core of vascular mesenchyme surrounded by an epithelium one cell deep. The individual cells were columnar in shape and possessed pale, granular cytoplasm and round or slightly elongated, hyperchromatic nuclei. The majority of the cells were uniformly granular, but a few in the extreme anterior portion of the plexus showed a slight degree of vacuolation. In these particular cells a small spherical vacuole was seen in the upper aventricular region, whilst the nucleus and its cytoplasmic investment lay in the lower ventricular portion. The transverse median fold in the roof of the fourth ventricle (see Text-fig. 2) was deeper than previously and the branching



Text-fig. 2. Rabbit, 17 days.

of the villi in its ventro-lateral walls was more pronounced. Further, the epithelial cells of the villi showed an even higher degree of vacuolation than in the preceding specimen. The anterior region of the medullary roof was even smaller in extent with the result that the median fold lay immediately behind the posterior lip of the cerebellum. The membranous area in the anterior portion of the roof showed even further reduction in size and was now relegated entirely to the upper part of the anterior wall of the median fold. Moreover, this area no longer showed abrupt demarcation from the surrounding epithelium but gradually merged into it. Its constituent cells had lost their flat appearance and were now low cuboidal in stature. The posterior membranous area was very extensive and at this stage occupied practically the entire posterior region of the roof.

The distribution of dye was similar to that in preceding stages, no extracerebral passage being evident.

18 *days' embryos.* The choroid plexuses of the lateral ventricles were extremely

well developed and their villi exhibited a higher degree of branching than previously. Moreover, the epithelial cells of these plexuses now showed a marked degree of vacuolation. In the majority of the cells the nucleus with its investing cytoplasm was relegated to the extreme ventricular periphery, whilst the aventricular portion of the cell was occupied entirely by a large vacuole. The morphology of the medullary roof was essentially similar to that of the preceding stage. The choroid villi of the fourth ventricle, however, were even more numerous, extending anteriorly below the cerebellum, and they showed a higher degree of branching. The epithelial cells of the villi were again markedly columnar in shape and their cytoplasm was extremely vacuolate. The membranous area in the anterior wall of the median fold showed even further regression than previously. The posterior membranous area, on the other hand, was even more extensive and now occupied the entire roof region posterior to the choroid plexus.

As in all earlier stages, the injected dye was wholly retained within the spaces of the central nervous system, no extra-ventricular spread being evident. A particularly dense accumulation of blue granules adhered to, and was co-extensive with, the ventricular surface of the membranous area in the posterior region of the medullary roof, but its individual cells were entirely free of the dye. No such condensation was evident below the vestigeal anterior membranous area.

Rats.

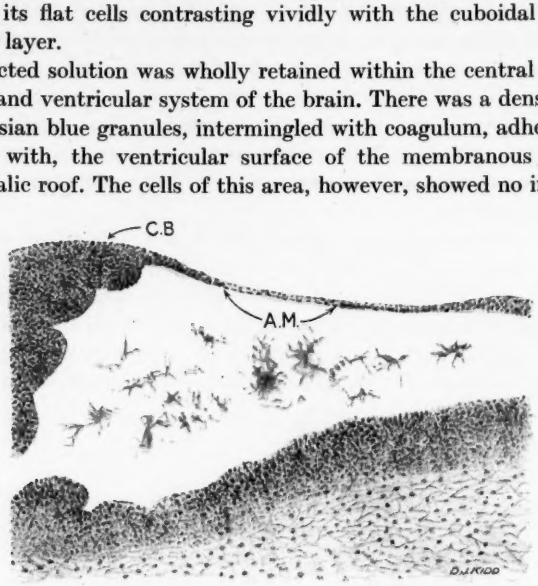
The series of rat embryos in which the central nervous system was injected ranged from 1·3 to 2·5 cm. in length. Two embryos were obtained measuring 0·6 cm., but owing to their size no injection was made into their central nervous system. These specimens were examined histologically, however, to determine the morphology of the brain at this stage.

Embryos measuring 0·6 *cm.* The wall of the brain stem was wholly ependymal in nature and was practically of uniform thickness. The roof of the fourth ventricle was typically triangular in shape, the wide base being directed anteriorly and the narrow apex posteriorly. This roof was continuous and unfolded throughout and consisted of a cuboidal epithelium one cell deep. The individual cells possessed pale, granular cytoplasm and round, clearly defined nuclei touching both the inner and outer cell walls. The nucleoplasm was pale in contradistinction to the intensely stained nucleoli and nuclear membrane.

Embryos measuring 1·3 *cm.* The wall of the brain stem was thicker than in the preceding stage and consisted of ependyma associated with a very narrow stratum of overlying nerve tissue. The roof of the third and fourth ventricles was in each case smooth and unfolded throughout and showed no indication of a choroid formation. The roof of the thalamencephalon consisted of an ependymal layer 3–4 cells deep. The individual cells were columnar in shape and possessed densely granular cytoplasm. Their nuclei were round or slightly elongated in a plane at right angles to the longitudinal axis of the body, and

possessed a deeply staining nuclear membrane and 2–3 nucleoli in their chromatin mesh. The roof of the fourth ventricle again consisted of a single layer of granular cuboidal cells. In the anterior region of this roof (see Text-fig. 3) a large, median oval area was distinguishable where the cells were extremely flat, consisting of a narrow elongated nucleus surrounded by a thin cytoplasmic investment. This area was abruptly delimited from the surrounding epithelium, its flat cells contrasting vividly with the cuboidal cells of the general roof layer.

The injected solution was wholly retained within the central canal of the spinal cord and ventricular system of the brain. There was a dense accumulation of Prussian blue granules, intermingled with coagulum, adhering to, and coextensive with, the ventricular surface of the membranous area in the myelencephalic roof. The cells of this area, however, showed no impregnation



Text-fig. 3. Rat, 1·3 cm.

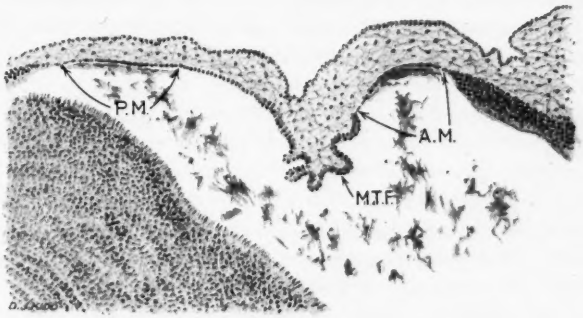
with the dye. Neither was there any spread of the injected solution from any other region in the brain stem.

Embryos measuring 1·5 *cm.* The roof of the thalamencephalon was somewhat thinner than previously but was still smooth and unfolded. At this stage the roof of the fourth ventricle was clearly divided into anterior and posterior portions by a shallow depression in the median transverse plane. The cells participating in the fold differed from the typical epithelial roof cells in that they tended to be more columnar than cuboidal and their nuclei oval rather than rounded. The membranous area in the anterior portion of the myelencephalic roof was larger than in the preceding specimens, extending from the cerebellar anlage to the median depression.

The injected solution was strictly confined within the spaces of the central nervous system, no extra-ventricular spread being evident.

Embryos measuring 1·7 *cm.* The thalamencephalic roof was much thinner than hitherto, consisting of a columnar epithelium one cell deep, and was

markedly undulating. The simple irregular folds so formed were invaded by the overlying mesenchyme and extended anteriorly for a short distance into the lateral ventricles via the foramina of Munro. The median transverse invagination in the roof of the fourth ventricle was deeper than in the previous stage (see Text-fig. 4) and its cells more columnar in shape. Moreover, its ventro-lateral walls showed a slight degree of folding but no true branching choroid villi were evident. The anterior portion of the myelencephalic roof was shorter in longitudinal section than formerly, owing apparently to encroachment by the developing cerebellum. The membranous area in this region now showed a slight reduction in extent and was partly incorporated into the anterior wall of the median fold. This area was still sharply delimited from the surrounding epithelium and its constituent cells retained their flat appearance. In the posterior region of the roof a small median, oval area was visible where



Text-fig. 4. Rat, 1·7 cm.

the cells showed a slight tendency to flattening, but their nuclei were still rounded or only a little elongated. This area was not sharply delimited from the surrounding epithelium but showed gradual mergence with it.

The injected dye was still retained within the central nervous system and was particularly condensed below the anterior membranous area in the roof of the fourth ventricle. No such condensation was seen below the slightly flattened posterior area, but merely a fine distribution of granules adhering to its ventricular surface.

Embryos measuring 1·8 *cm.* At this stage the undulations in the thalamencephalic roof were more pronounced than previously and the incipient choroid villi so formed extended forward for a considerable distance into the lateral ventricles. The median transverse fold in the roof of the fourth ventricle was more emphasized than in the preceding specimen and its ventro-lateral walls more markedly undulating, forming short unbranched incipient villi. The anterior portion of the myelencephalic roof was even shorter in extent, and the membranous area in this region of the roof was incorporated yet further into the anterior wall of the median fold. The area, however, was still sharply

delimited from the surrounding epithelium. The slightly flattened posterior membranous area was relatively much larger, but still showed gradual mergence with the remainder of the roof.

The distribution of the injected solution was similar to that in the preceding specimens, no extra-cerebral passage of dye being evident.

Embryos measuring 1·9 *cm.* These embryos presented the same general histological features as in the previous stage, but the median depression in the myelencephalic roof was deeper and its ventro-lateral walls showed a higher degree of folding. The anterior membranous area of this same roof showed even further reduction in size and was now wholly incorporated into the anterior wall of the fold. The posterior area on the other hand was relatively very large. At this stage it was abruptly demarcated from the surrounding epithelium and its constituent cells were extremely flat.

The dye was still confined within the spaces of the central nervous system and was now particularly condensed below the membranous area in the posterior region of the roof of the fourth ventricle. No such condensation was evident below the anterior area of this roof.

Embryos measuring 2·1 *cm.* In these specimens the villi in the thalamencephalic roof were very pronounced and, as they extended forward into the lateral ventricles, showed a slight degree of branching, forming an embryonic choroid plexus. In section each villus consisted of an axial core of loose-meshed vascular mesenchyme surrounded by an epithelium one cell deep. The cells were columnar in shape and possessed granular cytoplasm and round, well-defined nuclei. The majority of these cells were uniformly granular, but a few in the extreme anterior portion of the plexus showed a marked degree of vacuolation. The nucleus with its investing cytoplasm was relegated to the lower (ventricular) portion of the cell, the upper or aventricular region being occupied by a large spherical vacuole. The median transverse fold in the myelencephalic roof was very deep, extending almost to the floor of the ventricle (see Pl. I, fig. 4), and its constituent cells were markedly columnar. The incipient villi in its ventro-lateral walls were more pronounced than previously and were slightly branched. Many of the cells in the epithelium of the villi contained a large spherical vacuole in their aventricular portion, the nucleus and its cytoplasmic investment lying in the lower ventricular half. The membranous area in the anterior region of the fourth ventricle roof was even smaller than in the preceding stage and its border was no longer sharply defined but merged gradually into the surrounding epithelium. The constituent cells of this area had lost their flat appearance, being low cuboidal in stature, and their nuclei were now round or only slightly elongated. The posterior membranous area was even more extensive than previously, occupying almost the entire roof region behind the choroid plexus.

The injected dye was still confined within the central canal of the spinal cord and ventricles of the brain. No extra-cerebral spread was observed from any area in the brain stem.

Embryos measuring 2·3 *cm.* In these specimens the villi of the choroid plexuses of the brain showed an even higher degree of branching than previously and now the majority of their epithelial cells were vacuolate. The anterior membranous area in the medullary roof showed even further regression and was vestigeal in appearance.

The distribution of Prussian blue granules was similar to that of the preceding stage, no extra-ventricular spread being evident.

Embryos measuring 2·5 *cm.* At this stage the choroid villi of the plexuses were very numerous and highly branching, and the cells of their epithelium extremely vacuolate. The anterior portion of the medullary roof was very short in longitudinal section, consequently the median transverse fold lay immediately behind the cerebellar anlage. The vestigeal membranous area in this region of the roof had now vanished entirely. The posterior area was very extensive and occupied the entire roof region posterior to the choroid plexus.

As in all previous specimens, the injected solution was confined within the spaces of the central nervous system. At this stage the blue granules were heavily condensed below the membranous area in the posterior portion of the medullary roof, but the individual cells of this area were entirely free of the dye.

Guinea-pigs.

The present series of embryos in which injections were made into the central nervous system ranged from 1·6 to 3·2 cm. in length. One embryo measuring 0·4 cm. was available to us, but owing to its size the central nervous system was not injected. Nevertheless, this specimen was examined microscopically to determine the morphology of the brain at this stage.

Embryo measuring 0·4 *cm.* The wall of the brain stem consisted of a layer of ependyma only. The roof of both third and fourth ventricles was continuous and unfolded throughout and showed no indication of villus formation. The roof of the fourth ventricle was triangular in shape, the wide base being directed anteriorly and the narrow apex tapering into the spinal cord. This roof consisted of a single layer of cuboidal cells with granular cytoplasm and round, clearly defined nuclei. In its anterior region a small, median, oval area was distinguishable where the cells showed a slight tendency to flattening but their nuclei were still rounded or only a little elongated. This area was not clearly delimited from the surrounding epithelium but showed gradual mergence with it.

Embryos measuring 1·2 *cm.* The wall of the brain stem was thicker than in the preceding specimen and consisted of ependyma and a narrow stratum of overlying nerve tissue. The roof of the thalamencephalon consisted of a layer of ependyma only and still showed no indication of folding. The myelencephalic roof was also smooth and continuous throughout. The slightly differentiated median area in the anterior region of this roof was now sharply delimited from the surrounding epithelium and its constituent cells were very flat, the cytoplasm forming a thin investment around the narrow, elongated nucleus.

The injected dye was wholly retained within the central nervous system, no extra-ventricular spread being evident. The blue granules, intermingled with coagulum, extended in uniform distribution along the central canal of the spinal cord and in the cerebral ventricles. They showed no condensation associated with any particular area in the brain stem.

Embryos measuring 1·6 *cm.* The roof of the thalamencephalon was still unfolded but was thinner than in the previous stage, consisting of an ependymal layer 2–3 cells deep. The individual cells possessed pale granular cytoplasm and round or slightly elongated nuclei. The nucleoli and nuclear membrane were deeply stained in contrast to the pale nucleoplasm. The roof of the fourth ventricle (see Pl. I, fig. 5) was now divided into anterior and posterior regions by a slight invagination in the median transverse plane. This depression was invaded by the overlying mesenchyme and its constituent cells tended to be columnar rather than cuboidal. The membranous area in this roof was larger than in the preceding specimen and occupied the greater part of the roof region anterior to the median invagination. In the posterior portion of the roof a small, median, oval area was observed where the cells were low cuboidal in stature but their nuclei were still rounded or only a little elongated. This slightly differentiated area was not sharply defined but merged imperceptibly into the surrounding epithelium.

The injected solution was still confined within the central nervous system, but no longer exhibited the same uniformity of distribution as previously. A dense accumulation of blue granules, intermingled with coagulum, was seen adhering to the ventricular surface of the membranous area in the anterior portion of the myelencephalic roof. The individual cells of this area showed no impregnation with the dye.

Embryos measuring 1·8 *cm.* At this stage, the roof of the thalamencephalon presented a slightly undulating appearance, forming several irregular, shallow folds invaded by the overlying mesenchyme. The median transverse depression in the myelencephalic roof was deeper than previously and its constituent cells more markedly columnar. Its ventro-lateral walls exhibited a slight degree of folding but no true branching villi were apparent. The anterior portion of the roof was shorter in longitudinal section than in the preceding stage, owing to encroachment by the developing cerebellum. The median membranous area in this anterior region showed a slight reduction in extent and was now partly incorporated into the anterior wall of the median fold. It was still sharply delimited from the surrounding epithelium and its constituent cells retained their flat appearance. The slightly differentiated posterior area, on the other hand, showed considerable increase in size, exceeding the anterior area both in length and width. It still merged into the general roof layer and its cells were again low cuboidal in stature.

The injected dye was still retained within the central nervous system and was particularly condensed below the membranous area in the anterior portion of the fourth ventricle roof. No such condensation was evident below the

slightly differentiated posterior area, but merely a fine distribution of granules adhering to its ventricular surface.

Embryos measuring 1·9 *cm.* The invaginations in the thalamencephalic roof were more pronounced than in the preceding stage and extended anteriorly for a short distance into the lateral ventricles via the foramina of Munro. The median transverse fold in the roof of the fourth ventricle was more emphasized and its ventro-lateral walls exhibited a higher degree of undulation. The membranous area in the anterior region of this roof was smaller in extent than previously but its border was still clearly defined. The slightly flattened posterior area was relatively larger and again merged gradually into the surrounding roof epithelium.

The distribution of dye was similar to that of the preceding stage and the blue granules were still wholly confined within the central nervous system.

Embryos measuring 2 *cm.* These specimens presented the same histological picture as in the previous stage, but the median transverse fold in the medullary roof was much deeper. The invaginations in its ventro-lateral walls were now very pronounced, forming short, unbranched incipient villi. At this stage, the membranous area in the posterior region of this same roof showed slight increase in extent and was now abruptly demarcated from the surrounding epithelium. Its constituent cells were extremely flat, the cytoplasm forming a thin investment around the very elongated nucleus.

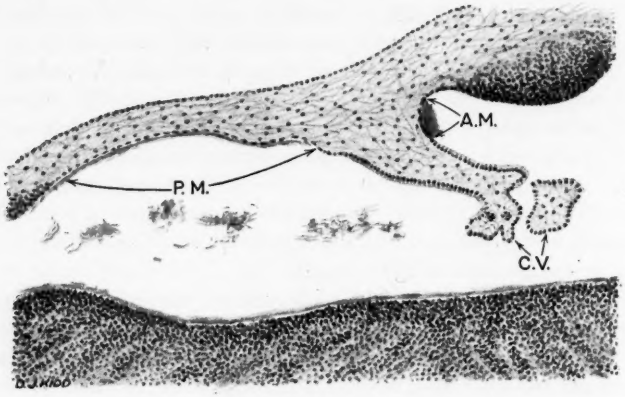
The distribution of injected dye resembled that of preceding stages, no extra-ventricular passage being observed.

Embryos measuring 2·3 *cm.* The folds of the thalamencephalic roof were more marked than hitherto and, as they extended forward into the lateral ventricles, showed a slight degree of branching, forming slender choroid villi. Each villus in section consisted of a central axis of vascular mesenchyme surrounded by an epithelium one cell deep. The individual cells were low columnar in shape and possessed pale granular cytoplasm and round or slightly elongated nuclei with a rich chromatin content. The median transverse fold in the medullary roof (see Text-fig. 5) was very pronounced, extending almost to the floor of the ventricle. The villi in its ventro-lateral walls were more numerous than previously and were now slightly branched, forming an embryonic choroid plexus. In section, each villus consisted of an axial core of mesenchyme containing minute capillaries in its intercellular meshes and surrounded by an epithelium one cell deep. The cells were markedly columnar and possessed pale, granular cytoplasm and slightly elongated, clearly defined nuclei. In a large number, the nucleus and its cytoplasmic investment were relegated to the lower or ventricular region of the cell, the upper aventricular portion containing a large spherical vacuole. The developing cerebellum had encroached on almost the whole of the anterior portion of the medullary roof with the result that the membranous area of this region was now wholly incorporated into the anterior wall of the median fold. This area showed even further reduction in size and was no longer sharply delimited from the sur-

rounding epithelium but merged gradually into it. Its constituent cells had lost their flat appearance and were now low cuboidal in stature. The posterior area, on the other hand, was very extensive and occupied practically the entire roof region behind the choroid plexus.

The injected dye was still strictly confined within the central nervous system, no extra-ventricular spread being evident. There was a dense accumulation of blue granules below the posterior area in the medullary roof, but the individual cells showed no invasion by the dye. No such condensation was evident below the vestigeal anterior area.

Embryos measuring 2·8 cm. In these specimens, the histological appearance of the brain was essentially similar to that of the previous stage, but the choroid villi of the lateral ventricles showed a slight degree of vacuolation.



Text-fig. 5. Guinea-pig, 2·3 cm.

Embryos measuring 3·2 cm. The choroid villi of the lateral ventricles were more numerous than previously and exhibited a higher degree of branching. The median transverse fold in the medullary roof was very pronounced, and the branching villi in its ventro-lateral walls constituted a well-developed choroid formation extending anteriorly below the cerebellum. The epithelial cells of the villi were markedly columnar and the majority of them were now extremely vacuolate. The anterior portion of the fourth ventricle roof was very short in section, consequently the posterior cerebellar lip lay in close approximation to the anterior wall of the median fold. The membranous area in this region of the roof was no longer visible, even as a vestigeal structure. The posterior area had developed to such an extent that it now occupied the whole of the posterior portion of the roof.

As in all preceding stages, the injected dye was wholly retained within the central nervous system, no extra-cerebral extension being evident.

To summarize: the sequence of events in the morphological development of

the roof of the fourth ventricle is strikingly similar in rabbit, rat and guinea-pig embryos. In all cases two membranous areas, one anterior and the other posterior, are present in this roof at various stages of intra-uterine life. Both areas are sharply demarcated from the surrounding cuboidal epithelium and consist of extremely flat cells with narrow, elongated nuclei. The anterior area is rapidly differentiated at an early period of development. It then undergoes a gradual regression and is finally obliterated, owing to encroachment by the enlarging cerebellum and choroid plexus. The posterior area, on the other hand, appears at a relatively later stage of development. It increases in size and eventually occupies the major portion of the medullary roof.

The first choroid villi to develop are those of the fourth ventricle. They originate in the ventro-lateral walls of a median transverse fold in the medullary roof, and rapidly assume a branching appearance. In earlier stages the epithelial cells of the villi are uniformly granular but as development proceeds they become vacuolate. The nucleus and its surrounding cytoplasm is relegated to the ventricular periphery of the cell, the aventricular portion containing a large spherical vacuole. The plexuses of the lateral ventricles originate as undulations in the thalamencephalic roof. The folds become increasingly marked and, as they extend forward into the lateral ventricles, form slender branching choroid villi. The epithelial cells of the villi are, at first, granular throughout, but later assume a vacuolate appearance.

In all embryos examined the injected solution remained entirely within the central canal of the spinal cord and ventricular system of the brain. An extra-cerebral passage of dye was never observed at any stage of development, either through the membranous areas in the medullary roof or through the epithelium of the choroid villi.

The stages at which the salient morphological changes occur in the different embryonic types are tabulated below. Naturally such stages are not comparable with each other. Moreover, the lengths given of rat and guinea-pig embryos are, for reasons already stated, no reliable criterion of age. They are quoted, however, since they serve as a very rough indication of the stage of development.

Embryo	Disappearance of anterior membranous area	Differentiation of posterior membranous area	Formation of choroid plexus of fourth ventricle	Formation of choroid plexus of lateral ventricles
Rabbit	17 days	15 days	16 days	17 days
Rat	2·1 cm.	1·9 cm.	2·1 cm.	2·1 cm.
Guinea-pig	2·3 cm.	2 cm.	2·3 cm.	2·3 cm.

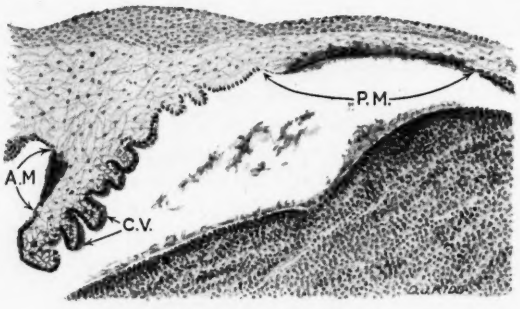
(*b*) *Effect of intra-vascular injections of a hypertonic solution on the distribution of a true solution injected into the central nervous system*

The present section is based on observations of rabbit, rat and guinea-pig embryos in which a 2 % saline solution was injected into the left ventricle of the heart. After a period of 4–5 min. had elapsed a 1 % double solution of

potassium ferrocyanide and iron ammonium citrate was injected into the central nervous system. Since a detailed account of the development of the medullary roof has been given in the previous section, observations relevant to the distribution of the injected double solution alone are recorded below.

Rabbits.

In embryos of 13, 14 and 15 days, the choroid plexuses of the brain were not fully differentiated. At these stages, the injected fluid was wholly retained within the spaces of the central nervous system. At 16 days, when the choroid plexus of the fourth ventricle had developed, the foreign solution had invaded the dorsal medullary mesenchyme (see Text-fig. 6). Both membranous areas in the myelencephalic roof functioned as the sites of fluid escape. Not only was there a dense accumulation of blue granules below these areas but in each case their constituent cells were impregnated with the dye. Blue precipitate was



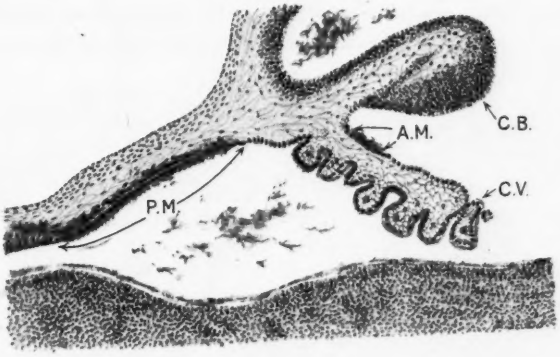
Text-fig. 6. Rabbit, 16 days.

also seen adhering to the ventricular surface of the epithelial cells in the choroid villi, but in this and in all succeeding stages the individual cells were entirely free of the dye. In embryos of 17 and 18 days, the posterior membranous area alone permitted of any fluid escape. There was a particularly heavy condensation of the injected solution adhering to its ventricular surface. Its cells were deeply impregnated with the dye and the mesenchyme immediately above this area contained in its intercellular spaces masses of coagulum intermingled with blue granules.

Rats.

In embryos measuring 1·6, 1·7 and 1·9 cm. no true branching choroid villi were apparent in the brain. At these stages the injected fluid was strictly confined within the central canal of the spinal cord and in the cerebral ventricles. In embryos measuring 2·1 cm. the choroid plexus of the fourth ventricle was fully differentiated (see Text-fig. 7). Accumulations of blue granules were seen adhering to the epithelium of the villi, but in these and in all later embryos

the cells showed no invasion by the dye. At this stage the foreign solution had extended into the overlying mesenchyme through the posterior membranous area in the medullary roof. A slight condensation of blue granules adhered to the ventricular surface of the vestigeal anterior membranous area, but its cells were entirely free of the dye. In embryos 2·2 and 2·4 cm. long the choroid plexuses of the lateral ventricles were well developed and the extra-ventricular passage of the injected solution was more marked than hitherto. Anteriorly the dye had spread over the cerebellar anlage and posteriorly for a short distance along the dorsal surface of the spinal cord.



Text-fig. 7. Rat, 2·1 cm.

Guinea-pigs.

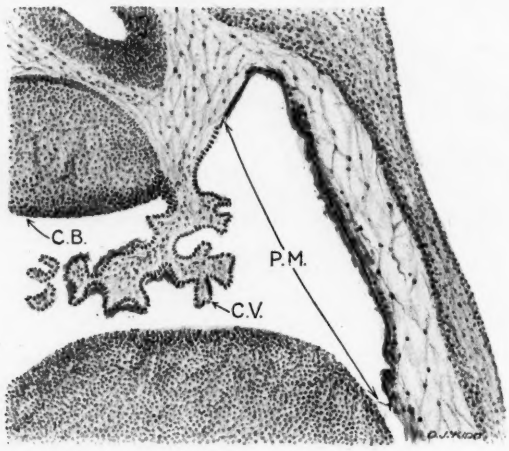
In embryos measuring 1·2, 1·5, 1·8 and 2·1 cm. no extra-cerebral passage of dye was evident; neither had the choroid plexuses of the brain developed. In embryos 3 cm. in length branching choroid villi were present in both lateral ventricles and in the fourth ventricle. At this stage a marked periaxial spread had occurred through the posterior membranous area in the medullary roof. There was a dense accumulation of blue granules adhering to the ventricular surface of this area. Its cells showed heavy invasion by the dye and the meshes of the overlying mesenchyme contained blue precipitate intermingled with coagulum. A marked condensation of blue granules was seen below the epithelial cells of the plexuses, but in these and in all subsequent specimens the individual cells were free of the dye. In embryos measuring 3·6 cm. the choroid villi of the cerebral ventricles showed a high degree of branching and the extra-ventricular spread of the injected solution was very pronounced (see Text-fig. 8). Anteriorly the dye had extended over the optic lobes and posteriorly along both dorsal and ventral surfaces of the spinal cord.

Thus, in embryos subjected to injections of a hypertonic saline solution an extra-ventricular passage of dye was observed, *but only at stages when the choroid plexuses of the brain were fully differentiated.* The injected fluid escaped

into the surrounding mesenchyme through the membranous areas in the roof of the fourth ventricle. Never, at any stage, was a spread observed through the epithelial cells of the choroid villi.

VI. APPENDIX

During the course of the present investigation, five mouse embryos came to hand incidentally. Their individual lengths (crown-rump measurement) were 1, 1·1, 1·3, 1·4 and 1·5 cm. respectively. No injections were made into the central nervous system of these embryos. They were examined histologically, however, to discover whether or not specialized membranous areas were present in the myelencephalic roof at any stage of development. Our observations are briefly recorded below.

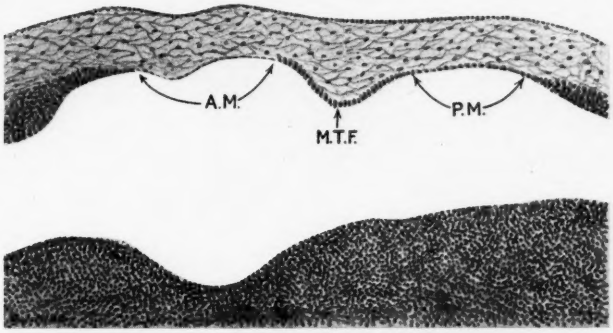


Text-fig. 8. Guinea-pig, 3·6 cm.

Embryo measuring 1 *cm.* The major portion of the roof of the fourth ventricle consisted of a cuboidal epithelium one cell deep. The individual cells possessed pale, granular cytoplasm and round, well-defined nuclei situated in the centre of the cell. This roof was divided into anterior and posterior regions by a shallow depression in the median transverse plane. The depression was invaded by the overlying mesenchyme and its constituent cells tended to be columnar rather than cuboidal. In the anterior region of this roof a large median, oval area was distinguishable where the cells were extremely flat, consisting of a narrow, elongated nucleus with a thin, granular, cytoplasmic investment. This area was abruptly demarcated from the remainder of the roof, its flat cells contrasting sharply with the cuboidal cells of the surrounding epithelium.

Embryo measuring 1·1 *cm.* This specimen presented very much the same appearance as the previous one, but the depression in the roof of the fourth ventricle was more emphasized.

Embryo measuring 1·3 *cm.* In this specimen (see Text-fig. 9) the invagination in the myelencephalic roof was deeper than previously, forming a definite fold, and its constituent cells were more markedly columnar. The membranous area in the anterior region of the roof was relatively larger, extending from the cerebellar anlage to the median transverse depression. In the posterior region a small, median, oval area was visible where the cells were low cuboidal in stature. Their nuclei, however, retained on the whole their rounded shape. This slightly differentiated area was not sharply delimited from the surrounding epithelium but showed gradual mergence with it.

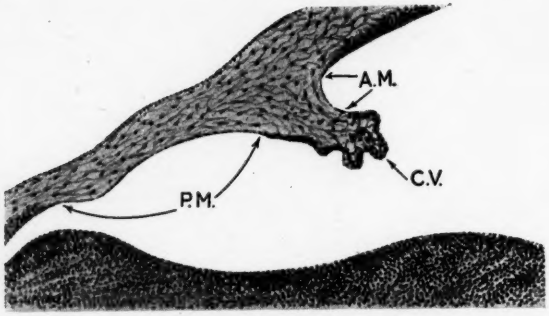


Text-fig. 9. Mouse, 1·3 cm.

Embryo measuring 1·4 *cm.* At this stage (see Text-fig. 10) the median transverse fold in the myelencephalic roof was very pronounced, extending almost to the floor of the ventricle, and its constituent cells were very clearly columnar. The ventro-lateral walls of this fold were strongly undulating and formed short, unbranched incipient villi supported by overlying mesenchyme. The majority of the epithelial cells in each villus possessed pale, uniformly granular cytoplasm, but in some cells one or two small vacuoles were seen lying close to the nucleus. The anterior portion of the fourth ventricle roof was considerably shorter in longitudinal section than in the preceding stage, consequently the median transverse fold lay immediately behind the cerebellar anlage. The membranous area in the anterior region of the roof was smaller in extent than previously and was now incorporated into the anterior wall of the median fold. This area, however, was still sharply delimited from the surrounding epithelium and its constituent cells retained their flat appearance. Practically the whole of the posterior region of the roof consisted of a now fully differentiated membranous area abruptly demarcated from the general roof epithelium and composed of flat cells with narrow elongated nuclei.

Embryo measuring 1·5 *cm.* This specimen presented the same general features as the previous one, but the villi in the ventro-lateral walls of the median fold showed a slight degree of branching, forming an embryonic choroid plexus. Further, a relatively larger number of the epithelial cells in the villi contained a spherical vacuole, still associated with the nucleus.

The small amount of material at our disposal naturally limited the scope of our investigation. Nevertheless, the following facts emerged clearly: (1) Anterior and posterior membranous areas are present in the medullary roof of mouse embryos during development. (2) A well-developed anterior area is present at a stage when the posterior area is only slightly differentiated. (3) When the posterior area is very extensive the anterior area is small. (4) The choroid plexus of the fourth ventricle originates from a median transverse fold in the medullary roof.



Text-fig. 10. Mouse, 1·4 cm.

Owing to insufficiency of data, a detailed history of the membranous areas is not given. The facts recorded above strongly suggest, however, that the sequence of events resembles that found in rabbit, rat and guinea-pig embryos.

VII. GENERAL SUMMARY AND DISCUSSION

Two membranous areas, one anterior and the other posterior, are present in the medullary roof of rabbit, rat, guinea-pig and mouse embryos at different stages of development. In all cases both areas are sharply delimited from the surrounding cuboidal epithelium and consist of extremely flat cells with narrow, elongated nuclei. From their microscopical appearance these areas are identifiable with similar structures recorded by Weed (1917) in pig and in human embryos, and by Cohen & Davies (1937) in the chick. Keegan (1917), investigating rabbit embryos, described two specialized areas in the roof of the fourth ventricle. The findings of the present investigation confirm his observations. Further, the two areas in the mammalian types investigated by

us appear to be homologous with the membranous area recorded by Flexner (1929) in the medullary roof of amblystoma embryos. In these latter, however, the area occupies almost the entire roof of the fourth ventricle without differentiation into anterior and posterior regions.

In rabbit, rat, guinea-pig and mouse embryos the anterior membranous area is a transitory structure and is obliterated early in development. Its disappearance is brought about by encroachment of the developing cerebellum and choroid plexus on the anterior portion of the medullary roof. The posterior area, on the other hand, is persistent and eventually occupies the major portion of the inferior choroid velum. Thus, the sequence of events in the history of the membranous areas in these types resembles very closely that found in pig, human and chick embryos.

Weed (1917) found that in pig embryos the first extra-ventricular spread of a 1 % double solution of potassium ferrocyanide and iron ammonium citrate injected into the central nervous system was coincident with the development of the choroid plexuses of the brain. Cohen & Davies (1937), using a 0·8 % concentration of the same salts, established a similar correlation in the chick. In pig and in chick embryos the membranous areas in the roof of the fourth ventricle functioned as the sites of fluid escape. The present research shows that the corresponding areas in rabbit, rat and guinea-pig embryos are impermeable to a 1 % solution of potassium ferrocyanide and iron ammonium citrate, even at stages when the choroid plexuses are well developed. Keegan (1917) records a similar observation also in rabbit embryos. Thus, it appears from our own findings and from those of other investigators, that the specialized areas in the medullary roof of various animals show a specific difference in permeability.

After intra-vascular injections of a hypertonic saline solution, the membranous areas in our material were rendered permeable to the 1 % double solution, but only at stages when the choroid villi of the brain had developed. Prior to differentiation of the plexuses an extra-ventricular passage of dye was never observed. Therefore, under such experimental conditions, there appears to be a definite relationship between the developing choroid plexuses and the first periaxial spread of fluid injected into the central nervous system. A similar conclusion reported by Weed (1917) in pig embryos and by Cohen & Davies (1937) in the chick is thus substantiated.

After increasing the salt content of the blood in various adult Mammalia Nañagas (1921) and Weed (1922) found that the epithelial cells of the choroid villi take no part in the intra-ventricular absorption of the cerebrospinal fluid. On the other hand, Foley (1923) and Forbes *et al.* (1928) state that under the influence of intravenous injections of a hypertonic salt solution the choroid plexuses absorb fluid from the ventricles. Our own findings in different embryonic Mammalia agree with those recorded by Nañagas (1921) and by Weed (1922) in adults. In rabbit, rat and guinea-pig embryos subjected to intra-vascular injections of a hypertonic saline solution, a passage of dye through the choroid plexuses was never observed at any stage of development.

During the intra-uterine life of these types the epithelium of the choroid villi under both normal and experimental conditions functions as a physiological barrier to the passage of fluid from the cerebral ventricles.

VIII. CONCLUSIONS

1. Two membranous areas, one anterior and the other posterior, are present in the medullary roof of rabbit, rat, guinea-pig and mouse embryos at different stages of development. In all cases the anterior area is a transitory structure and disappears early in intra-uterine life. The posterior area, on the other hand, persists and eventually forms the major portion of the inferior choroid velum.

2. In all stages of development the two areas in rabbit, rat and guinea-pig embryos are impermeable to a 1 % double solution of potassium ferrocyanide and iron ammonium citrate injected under normal conditions into the central nervous system. Under the influence of a hypertonic saline solution in the blood stream, they become permeable and permit of the extra-cerebral passage of dye from the fourth ventricle, but only when the choroid plexuses of the brain are differentiated. Therefore, under such experimental conditions a correlation is established between the development of the choroid villi and the first periaxial spread of a true solution injected into the central nervous system.

3. During embryonic life, the epithelium of the choroid plexuses under both normal and experimental conditions is a functional barrier to the passage of a true solution from the cerebral ventricles.

We wish to thank Mr F. Beckwith for taking the photographs. The text figures are the work of Mr Douglas J. Kidd, medical artist.

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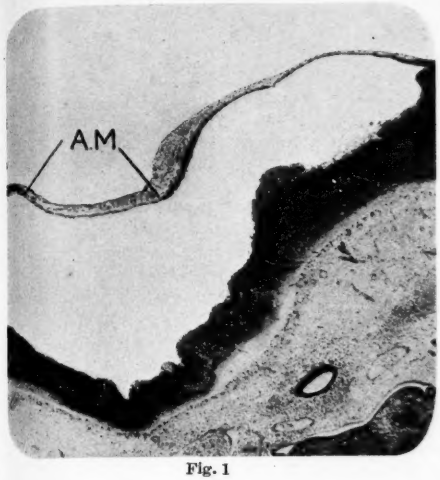

Fig. 1

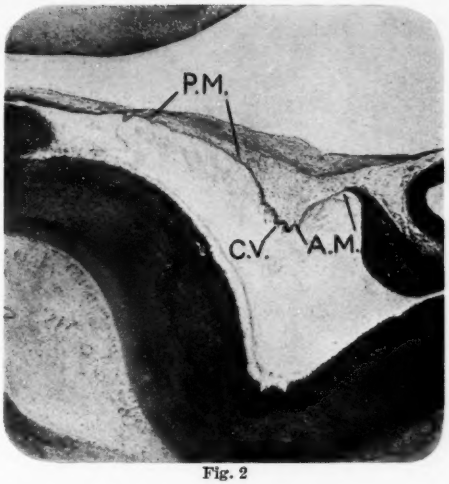

Fig. 2

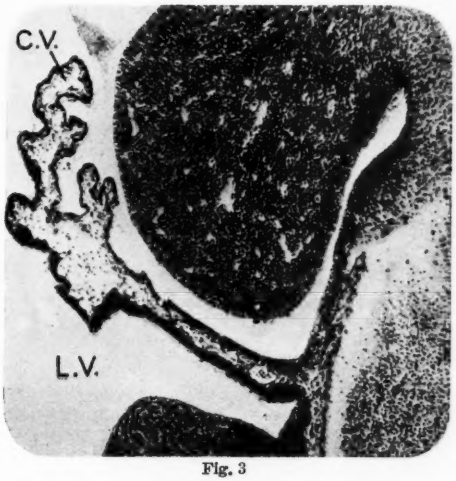

Fig. 3

Fig. 4

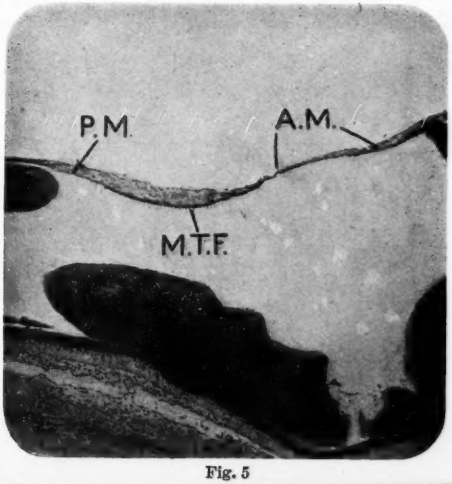

Fig. 5

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EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 13 days' rabbit embryo. In this specimen an injection of a 1% double solution of potassium ferrocyanide and iron ammonium citrate was made into the central nervous system. (Acid formaldehyde: Ehrlich's haematoxylin and eosin.) ×27.

Fig. 2. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a 15 days' rabbit embryo injected as in specimen illustrated in fig. 1. (Acid formaldehyde: Ehrlich's haematoxylin and eosin.) ×27.

Fig. 3. Photomicrograph of a longitudinal section of the right cerebral hemisphere in a 17 days' rabbit embryo injected as in specimen illustrated in fig. 1. (Acid formaldehyde: Ehrlich's haematoxylin and eosin.) ×72.

Fig. 4. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a rat embryo measuring 2·1 cm. and injected as in specimen illustrated in fig. 1. (Acid formaldehyde: Weigert's haematoxylin and eosin.) ×45.

Fig. 5. Photomicrograph of a longitudinal section of the roof of the fourth ventricle in a guinea-pig embryo measuring 1·6 cm. and injected as in specimen illustrated in fig. 1. (Acid formaldehyde: Weigert's haematoxylin and eosin.) ×36.

ABBREVIATIONS

A.M. Anterior membranous area.
C.B. Cerebellum.
C.V. Choroid villi.
L.V. Lateral ventricle.
M.T.F. Median transverse fold.
P.M. Posterior membranous area.

A RARE MALFORMATION OF THE HAND

By R. S. STACEY

From the Royal College of Medicine, Baghdad

Congenital abnormalities of the hands are common and have been classified into a number of well-established groups. The case reported here is described because it has features in common with one of these groups but differs from it in certain important respects.

The patient is a somewhat undersized male Arab from Mosul, who was admitted to the Royal Hospital, Baghdad, with lobar pneumonia. His right hand is very much smaller than his left and this difference is largely, though not entirely, due to the extreme shortness of the second phalanx of each of the fingers of that hand with the exception of that of the thumb. The fingers are partly webbed, the skin extending to within half an inch of the proximal interphalangeal joint. These changes can be seen in the accompanying photographs. There is no fusion of the phalanges but flexion at the interphalangeal joints of the four fingers is limited. The patient uses his right hand for writing but for purposes where more force is required he uses his left, which is normal.

The right arm is, throughout, slightly smaller than the left, both in the lengths of the bones and in the size of the muscles. Measurements are given below. All muscular power in this limb is diminished, but there is no evidence of any lesion of the nervous system. The tendon reflexes are normal. The smallness of the arm may be due to similar factors as those responsible for the changes in the hand but are of such a degree as might result from disuse due to the relative uselessness of the hand for manual work.

The legs are equal and symmetrical and there is no malformation of the toes. The Wasserman reaction is negative and there are none of the stigmata of congenital syphilis.

The right hand is stated always to have been as it now is, and it is not possible to ascertain when the deformity was first noticed. The patient's parents are dead but their hands are said to have been normal. He has six children, all of whom have been examined; none show any anatomical abnormality, and questioning, both of the patient and of his family, has failed to bring to light any evidence of any similar condition in any of his relatives.

Measurements

Height: 155 cm.
Sitting height: 86 cm.
Arm (acromion to lower end of radius): right, 52 cm.; left, 54 cm.
Upper arm circumference: right, 18 cm.; left, 19 cm.
Forearm circumference: right, 18 cm.; left, 19·5 cm.

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Comment. The deformity is of the type described by Drinkwater (1912) as "minor-brachydactyly" in that the main abnormality is the extreme shortness of the middle phalanges. The shortness of stature also conforms with the usual findings in this condition. It differs from it, however, in two important respects:

(1) In this case the changes are confined to one hand, while in the other cases which have been described, so far as I am able to ascertain without the facilities of an extensive reference library, both hands and both feet have been affected.

(2) This is a sporadic case without evidence of any inheritance, while brachydactyly behaves as a mendelian dominant. Since the parents have not personally been examined and the progeny are limited to one generation, this point is open to question. Hasselwander, cited by Lewis (1909), has described a case in which a brachydactylous individual arose from normal stock, but this mutation was transmitted in later generations.

The interest in this case lies in the appearance of a malformation, usually hereditary, being not hereditary in an individual in whom, moreover, it is unilateral instead of bilateral.

My thanks are due to Dr A. C. Norman for the X-ray of this case.

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EXPLANATION OF PLATE I

X-ray pictures of the hands. Notice the shortness of the second phalanx in digits ii, iii, iv and v in the X-ray on the left. The photographs illustrate the webbing.

THE PREPUTIAL GLANDS OF BRITISH MURIDAE

By L. H. JACKSON

From the Department of Zoology, University College of North Wales, Bangor

During an investigation of the anatomy of the reproductive organs of the male *Apodemus sylvaticus* it was noticed that the preputial glands of the adult were associated with a hair follicle. As this condition appears never previously to have been described in any animal with large compact preputial glands, it was thought worth while to examine the preputial glands of several species of rodent, to determine the extent of its occurrence. This paper is an account of the condition of the preputial glands in the species *Apodemus sylvaticus* (L.), *Mus musculus* (L.), *Rattus rattus* (L.), *R. norvegicus* (Erxleben), *Clethrionomys glareolus* (Miller), *Microtus agrestis* (Bellamy), *Arvicola amphibius* (L.) and *Ondatra zibethica* (L.).

Compact preputial glands are absent in *Oryctolagus*, *Cavia* and *Sciurus* (Disselhorst, 1904) and in *Cricetus* and *Muscardinus* among the rodents. They are also lacking in those Insectivora and Chiroptera which have been examined, viz. *Sorex* (Brambell, 1935), *Microsorex*, *Neomys*, *Talpa* and *Erinaceus* (Disselhorst, 1904), and the *Pipistrelle* bat.

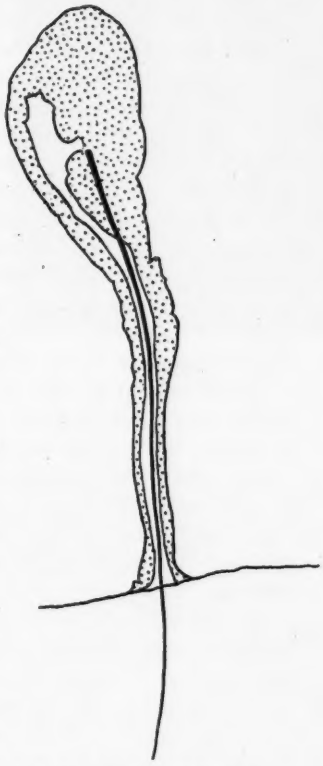
The preputial glands of *Apodemus sylvaticus* lie closely applied to the sides of the penis near its tip. They are compact and club-shaped, and about 2·5 mm. long at maximum development, with a duct 4 mm. long opening into the preputial sac. The glands become very minute during winter anoestrus. They differ in shape from those of the other mice and voles.

The preputials are enlarged sebaceous glands opening into a follicle, the hair of which traverses the duct and projects from its mouth (text-fig. 1). In Plate I, figs. 1 and 4, the hair follicles are seen in transverse section, surrounded by the tissue of the gland. The glands of three specimens were examined. In each case the gland of one side contained one hair, while that of the other contained two.

Hairs were also found in the glands of *Rattus rattus* and *Mus musculus*, including the albino variety of the latter. The preputial glands of the black rat are club-shaped, about 15 mm. long including the duct, and 3–4 mm. broad. One preputial of an adult black rat was found to contain three hairs, with their follicles buried in the body of the gland and their shafts traversing the duct, the arrangement being very similar to that in *Apodemus*. In another specimen, each gland also contained three hairs.

The house mouse (*Mus musculus*) has preputial glands of the flattened shape found in *Clethrionomys*, but more compact. The glands are larger in

proportion to the size of the animal than those of either *Apodemus* or the voles, being 8 mm. long × 5 mm. broad in an old specimen. Plate I, fig. 3, shows the two hairs found in the preputial gland of one side of a specimen of the house mouse; the follicle of one of the hairs is seen in lateral view, lying in the plane of the section. The figure also shows the absence of pattern in the preputial hair compared with the general body hairs. Two hairs were also found in the preputial gland of the other side of the same specimen.



Text-fig. 1. Preputial gland of *Apodemus sylvaticus*. × 14.

The preputial glands of the white mouse are similar to those of the house mouse, and are shown in Plate I, fig. 2. Two specimens were examined, one of which was found to have many scattered hair follicles in each gland, while the other had but three hairs on each side.

In the definitive condition the preputial glands of the species *Clethrionomys glareolus*, *Microtus agrestis*, *Arvicola amphibius*, *Ondatra zibethica* and *Rattus norvegicus* do not contain hairs. The preputial glands of *Clethrionomys glareolus* (Rowlands, 1936) are much larger and more diffuse than those of *Apodemus*, being about 6 mm. long, with a duct 7 mm. long. They are flattened and leaf-like in shape, and extend forwards over the hinder part of the ventral wall of the abdomen. In this they differ from those of *Apodemus* and resemble more those of *Mus musculus*. The glands of *Clethrionomys* are filled with large cells with conspicuous nuclei, giving them a coarse granular appearance in contrast to those of *Apodemus*. It appears probable that the secretion of the glands is formed by the breakdown of these cells.

Microtus agrestis has preputial glands much smaller than those of *Clethrionomys*, being only 6 mm. long including the duct in the adult specimen examined. They are much more like those of *Apodemus* in microscopic appearance and shape, but less clubbed.

The preputials of *Arvicola amphibius* are similar in shape and proportionate size to those of the previous species.

The musk rat (*Ondatra zibethica*) has the preputials modified into scent glands. They are compact and very large. The gland in the single specimen examined was some 35 mm. in length.

Both *Rattus norvegicus* and its albino variety have club-shaped preputial glands very like those of *Rattus rattus* in size and shape, but no trace of hairs was found in the glands of either variety of this species.

DISCUSSION

The derivation of the preputial glands of many animals from skin glands of the sebaceous type has been assumed since the time of Leydig (1850); and the condition in *Oryctolagus* and *Cavia*, in which the only preputial glands are small scattered sebaceous glands opening into hair-follicles (Disselhorst, 1904) has been supposed to be primitive. The glands in these animals however are very different from the greatly enlarged, compact glands so characteristic of the rats, mice and voles. Therefore the discovery that the glands of some of the latter retain their connexion with the hair and its follicle in the definitive condition is of some interest, and confirms the belief that these two types of preputial gland are homologous. The case is similar to that of the mammary glands of Eutheria, which are at first connected with hair follicles, which usually disappear during development (Bresslau, 1920).

It will be noticed that, of the species investigated, the hairs were found in all the mice and some of the rats, but in none of the voles—i.e. in part of the subfamily Murinae but not in the Microtinae.

The presence of the hairs in a species would appear to prove that the gland in question is of sebaceous derivation. It is therefore of particular interest that *Rattus rattus* clearly possesses this follicular structure, as it has been doubted whether the preputial glands of rat have been so derived (Disselhorst, 1904).

It was noticed that the hairs in the preputial glands lack the medullary pattern usually found in the general body hairs of the species. This applies to all the species in which the hairs occur. The hairs on the surface of the prepuce however are also often devoid of pattern.

It will be noticed that the number of hairs present (indicating presumably the number of follicles concerned in the origin of the gland) ranges from one or two in *Apodemus* to many in *Mus musculus*; and that the number shows individual variation in some species.

SUMMARY

In the rodents *Apodemus sylvaticus*, *Rattus rattus* and *Mus musculus* (including the albino variety) the preputial glands are of sebaceous origin, and in the definitive condition retain their connexion with the hair follicles from which they arose, the hairs emerging from the mouth of the duct and projecting from the surface of the prepuce.

The rodents *Rattus norvegicus*, *Rattus n. albinus*, *Microtus agrestis*, *Clethrionomys glareolus*, *Arvicola amphibius* and *Ondatra zibethica*, all of which have enlarged compact preputials similar to the above species, do not show this condition.

Fig. 1.

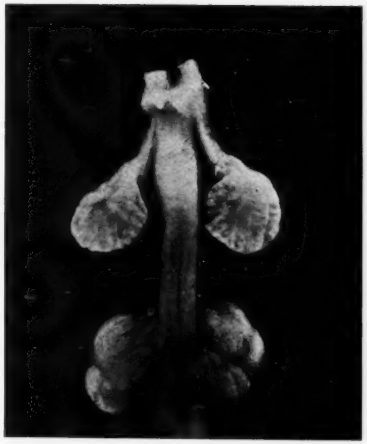


Fig. 2.

Fig. 3.

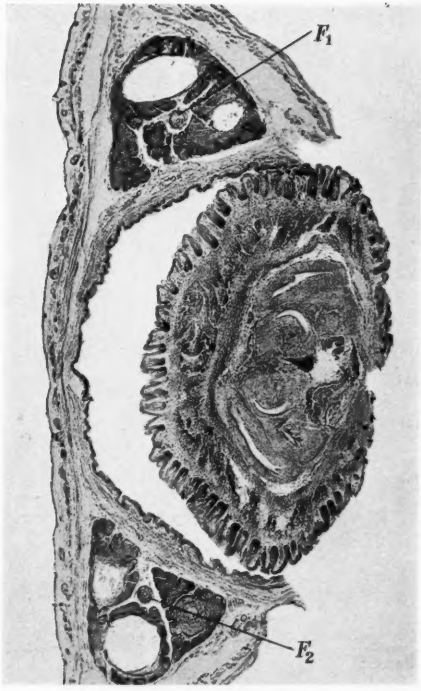


Fig. 4.

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I have to thank Prof. F. W. Rogers Brambell, Dr A. S. Parkes and Miss K. Hall for supplying me with some of the material, and the former especially for his interest and advice. I have also to thank Mr Tom Warwick for providing material of the water vole and musk rat.

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EXPLANATION OF PLATE I

Fig. 1. Photomicrograph of a transverse section through a preputial gland of *Apodemus sylvaticus*, showing the hair follicle surrounded by glandular tissue. ×210.

Fig. 2. Photograph of the ventral aspect of the bulbar complex and terminal portion of the urethra of the white mouse, showing the preputial glands and their ducts. ×2·5.

Fig. 3. Photomicrograph of a transverse section of the preputial gland of *Mus musculus*, showing a hair follicle, with the shaft of the hair entering the lumen of the duct. A portion of another hair is seen in the lumen. ×167.

Fig. 4. Photomicrograph of a transverse section of the penis of *Apodemus sylvaticus*, showing the preputial glands, each with its hair follicle F_1 and F_2. ×23.

THE FATE OF THE HUMAN PREMAXILLA

By FREDERIC WOOD-JONES

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THE fate of the osseous premaxillary element in the human facial skeleton is a subject around which an extensive literature has grown up. This literature has been summarized more than once in readily accessible English publications. In 1929 the present writer dealt with the subject and in 1935 Mr Astley-Montague devoted a great deal of research to this aspect of the question, and published an exhaustive series of abstracts from the literature of all periods. In 1937, Dr Edwin Johnson published in this *Journal* an abstract of his investigations into the fate of the premaxillary element in the mammalian facial skeleton. Johnson's conclusions had already been embodied in an unpublished thesis before the appearance of Astley-Montague's papers and he had already appreciated the importance of Callender's 1869 publication before Astley-Montague called attention to this much neglected contribution. Callender was the first to postulate that the maxillae overgrew the premaxillae on the human face. In 1914, unaware of the previous work of Callender, the present writer published as his conclusion that the human premaxilla "becomes overgrown by the facial portion of the maxilla". From this standpoint, Johnson started his exhaustive investigation and showed, almost dramatically, how the narial margins were formed in man. He dwelt especially upon the "*exclusively human*" character of this portion of the facial skeleton, and gave references to the essential literature. Dr Douglas Derry's paper (which cites no previous work) in the current issue of the *Journal of Anatomy* (vol. LXXII, pt. II, Jan. 1938, pp. 294–8), is interesting as confirming Johnson's amply documented findings, which in turn agreed with the less precise, but identical, conclusions of the present writer in 1914 and of Callender in 1869.

ANATOMICAL NOTES

FURTHER STUDIES ON THE ORIGIN OF THE FIRST DORSAL INTEROSSEOUS MUSCLE OF THE FOOT FROM THE TENDON OF THE PERONAEUS LONGUS

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The first dorsal interosseous muscle of the foot is described in the standard text-books as arising by medial and lateral heads from the adjacent sides of the first and second metatarsal bones. In 1933 the writer described a variation of this origin. In a series of 27 feet from 15 male bodies, the precise origin of the muscle was examined. The origin of the lateral head was found to be as commonly described, but in 20 dissections out of 27 (or 11 subjects of the 15 dissected) the medial head was found to arise by means of a tendinous slip from the peronaeus longus tendon as it (the tendon) passed along the peronaeal sulcus on the plantar surface of the cuboid bone. In these dissections the attachment of the medial head to the first metatarsal bone was by means of loose connective tissue only. The seven feet, where no tendinous origin was found, showed a direct muscular origin for the medial head from the proximal part of the medial side of the first metatarsal bone. The tendon of origin was always enclosed in a synovial sheath continuous with that enclosing the peronaeus longus tendon. Its relation was dorsi-lateral to the peronaeus longus tendon.

Since this first report was made records have been kept of the dissections of the feet made in 1934 and 1937. The origin of this muscle was found to be as described in 1933 in many cases. Toldt's *Atlas of Human Anatomy* contains an illustration in which the medial head of the muscle appears as if it had an origin from the tendon of the peronaeus longus, but no attention is drawn to it.

Bryce mentions that the first dorsal interosseous muscle receives fibres of origin from the peronaeus longus tendon at its insertion. Which head receives these extra fibres is not made clear. No references other than these have been found in the literature available.

In 1934 the origin of the first dorsal interosseous muscle was noted in 24 feet from 13 male subjects. The origin by means of a tendinous slip as described above was present in both feet in 4 subjects or 8 feet. It was absent on both sides in 5 subjects or 10 feet. It was present on the right side in 1 subject. It was present on the left side only in 1 subject. It was present on the right side in 1 subject where there was no dissection of the left foot. It was absent on the right side in a subject having one extremity amputated. The tendinous origin was therefore present in 11 feet out of 24 dissected.

In 1937 the origin of this muscle was carefully defined in 24 feet from 12 male subjects. The tendinous origin was present on both sides in 6 subjects or 12 feet. It was absent on both sides in 4 subjects or 8 feet. It was present on the right side only in 2 subjects. It was present on the left side only in 1 subject. The tendinous origin was therefore present in 15 feet out of 24 dissected.

In the latter group (1937) the tendon of the peronaeus longus muscle was noted to give a tendinous slip of origin to the oblique head of the adductor hallucis muscle on both sides in 3 subjects out of 12 examined. In one foot the tendon of the peronaeus longus divided into two equal parts, one was inserted in the usual manner, the other was inserted into the tuberosity of the fifth metatarsal bone.

As described previously (1933) the tendon of the muscle taking origin from the tendon of the peronaeus longus varies considerably in size but is defined easily, when present, by retraction of the tendon of the peronaeus longus. The average student fails to do this. The smaller tendon is enclosed in a mucous sheath derived from that enclosing the larger tendon and is continuous with it.

Table I. *Tendinous origin of first dorsal interosseous muscle*

	1933	1934	1937	Total
No. of subjects	15	13	12	40
No. of feet dissected	27	24	24	75
Tendinous origin	20	11	15	46
Muscular origin	7	13	9	29
Per cent tendinous	74·0	45·8	62·5	61·3

SUMMARY

1. A second report on the origin of the first dorsal interosseous muscle of the foot is presented. It is based upon a larger number of dissections and should be of more value than the first report of 1933.

2. In 75 feet from 40 subjects the medial head of this muscle was found to take origin by means of a tendinous slip from the tendon of the peronaeus longus in 46 feet or 61·3 %.

3. This type of origin is considered to be a frequent variation and not an anomaly. As such, it should be described in standard text-books.

ACKNOWLEDGEMENT. The writer wishes to express his sincere thanks to Prof. J. Beattie for the reference to Bryce and his valuable advice.

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BILATERAL ANOMALY OF THE LARYNGEAL SACCULE

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THE case under review is that of a dissecting room subject. No history was available. The individual was a male, 82 years of age. The abnormality was noticed by the students in the ordinary course of their work. It consisted of enlarged laryngeal saccules on both sides of the neck.

Right side

A sac-like structure was found in the right side of the neck just above the upper margin of the thyroid cartilage. It was overlapped by the infrahyoid muscles and it was found to end blindly. On freeing the structure from its bed it was observed

Fig. 1. Photograph of neck region in dissecting room subject. It shows the laryngeal saccule outside of the thyrohyoid membrane. The sac is picked up by the forceps and is held off the membrane.

to pass through an aperture in the lateral part of the thyrohyoid membrane. Following the structure on the deep surface of the membrane, it opened into the anterior part of the corresponding sinus of the larynx. From its origin it was directed forwards and upwards and on piercing the thyrohyoid membrane it was reflected laterally on its superficial surface. The length of the sac on the right side, from base to tip, was 6 cm. The part outside the membrane measured 3·2 cm. The width varied from 0·5 to 1·0 cm.

Left side

The laryngeal saccule on the left side was also found to be lengthened, although it did not traverse the thyrohyoid membrane. It passed from its base at the anterior end of the left laryngeal sinus, upwards, forwards, and laterally for a distance of 4·5 cm. The blind extremity of the sac was situated on the deep surface of the lateral part of the thyrohyoid membrane. The width varied from 0·3 cm. at its root to 0·5 cm. near its tip. No evidences of laryngeal pathology were found and the structures were freed with ease from the surrounding tissue.

Discussion

Enlargement of the saccule of the laryngeal sinus was first recorded by Bennet in 1865. The case which he reported was similar to the present one except the two sacs which he described were of almost equal size. Prof. Sclavunas (1904) of Athens reviewed the literature of his time and added three more cases to those which had already been reported.

Thirteen cases have been described thus far, most of which were discovered in the dissecting room; six showed one-sided enlargement and in seven the anomaly was present on both sides. Of the seven bilateral cases five showed a larger sac on one side than on the other.

The anomaly has been recognized clinically. In a recent Murphy oration, Wheeler (1933) describes such a case:

> The patient was a man aged 24 years, who suffered all his life from a painless bulge in the right submaxillary region. This swelling was tympanitic and under pressure disappeared. The swelling moved and protruded with deglutition and coughing. The right side of the tongue and floor of the mouth were enlarged. On the posterior third of the dorsum of the tongue on the right side, there was a soft conical projection about the size of a lump of sugar. The tongue could not be protruded. Any attempt to do so resulted in deviation of the entire organ on the right side. Speaking was difficult. It was owing to the fact that his friends could not understand what he said that he came to hospital to seek advice. The swelling on the tongue and the swelling in the submaxillary region were connected. If the one was pressed upon, the other enlarged. It was obvious that the swellings were not solid, nor did they contain fluid. The introduction of a hypodermic needle into the protrusion on the tongue gave no information. After the injection of lipiodol through the needle X-ray photographs confirmed the fact that the tongue swelling and the submaxillary swelling were connected.

This case cited by Wheeler was undoubtedly one of congenital laryngocoele and its great interest lies in the extension of the swelling into the base of the tongue.

The abnormality which has been described resembles in certain respects the larger and more extensive laryngeal saccules of some anthropoids. According to Symington (1914) these sacs may extend downwards in the neck superficial to the clavicle, between the sternal and clavicular heads of the pectoralis major muscle to end blindly in the axilla.

I wish to acknowledge my indebtedness to Prof. J. L. Jackson for his criticism and suggestions.

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REVIEWS

The Dissection and Study of the Sheep's Brain. 1st Edition by JAMES WILKIE, B.Sc., Department of Biology, Guy's Hospital Medical School. With Foreword by PROFESSOR T. B. JOHNSTON. (Oxford University Press. London: Humphrey Milford.) 1937. pp. 95, incl. index. 53 Figs. 6*s*.

Sheeps' brains are cheap and easy to procure, and much can be learnt from their study. Therefore this textbook will be useful to students who wish to make acquaintance with the mammalian Brain.

Civilisation and Disease. By C. P. DONNISON, M.D. Lond., M.R.C.P. Lond. (London: Baillière, Tindall & Cox.) 1937.

This is an extremely interesting book. The author has meditated upon the diseases met with in medical practice amongst the natives of Africa and the inhabitants of England. His own observations, supplemented by those made by other doctors with similar opportunities, have enabled him to arrive at a computation of the frequency with which the different diseases occur amongst uncivilized races. He has presented a table setting out the frequency with which the different diseases are encountered in practice in such places as Kenya, Tanganyika, etc., and the frequency with which the same diseases are a cause of death in England. Diabetes, exophthalmic goitre, hyperpiesis, and peptic ulcer are the organic diseases which are prevalent in a civilized community, but are rare, if not quite unknown, amongst a primitive people. In addition, the native almost never suffers from any of the psychoneuroses.

The author considers our knowledge of the causation of these organic diseases and comes to the generally acceptable conclusion that they start from a functional disorder of the autonomic and endocrine systems of the body. The conception of the body as a psychosomatic unit takes shape as the book proceeds and is important for the view-point which the author finally reaches.

A consideration of the psychoneuroses involves a critical review of the well-known, even if not well-understood, theories of abnormal psychology. The calm objective spirit of the book is apparent in the discussion of the writings of Freud, Jung, etc. The author prefers the Adlerian view of the neuroses partly because he feels that its simplicity of statement is indicative of a greater nearness to the truth, because of its more teleological character and its greater insistence on the influence of the environment. Inevitably such a discussion leads to a presentation of theories of the origin of civilization.

Perhaps the survival of *Homo sapiens* and the disappearance of other types of man, certainly all progress to a higher cultural level, have depended on the replacement of egoistic individualistic urges by others of a more co-operative nature. A more efficient social activity demands a more difficult social adjustment, and the need and the difficulty of this adjustment constitute the real difference between the civilized and the uncivilized life. Of the factors leading to an increase in the social sentiments, social heritage or education is one of the most important. The diseases which are incident to any advance in cultural level arise from a faulty education in the wider sense.

It is not to be supposed that the native in escaping from the diseases of civilization enjoys any undue immunity from destructive forces. As a matter of fact his life is much shorter than that of his civilized brother. The lack of any sort of routine in

the rearing of native infants probably causes them as much frustration as the children of a higher social heritage. The native is almost never alone. He is therefore more suggestible, more likely to act from ill-defined group motives. He never has to worry about what people will think. He always knows. There are no symbols—fences, introductions, small talk—of separateness in native life.

These few comments make a very inadequate sketch of the erudition and the contemplation the author has brought to bear on the fascinating problem of civilization and disease.

Surface and Radiological Anatomy (*for students and general practitioners*). By A. B. Appleton, W. J. Hamilton and I. C. C. Tchaperoff. (Cambridge: W. Heffer and Sons.) 1938. Pp. 311, with 338 illustrations. Price 15*s.* net.

This interesting book will be welcomed alike by anatomists, radiologists, students and practitioners. It provides a concise yet comprehensive account of the anatomical features which are accessible to examination in the living subject.

There is a useful short introduction on radiological technique, the structure of the skin, and the group action of muscles. Each of the recognized regional divisions of the body is then dealt with, the clinical applications of the subject being always kept in the foreground. The general plan of each section is to consider the superficial features first, then the muscles, then the bones and joints with a radiographic study of their movements, and lastly the vessels, nerves and lymphatics. In the case of the trunk, the viscera are also examined in turn—where possible—by the methods of physical examination, radiology, digital exploration and by the use of special instruments. The radiological studies illustrate once more the well-recognized fact of variation in the positions of many organs and show that they cannot be adequately represented by the customary fixed surface markings. In the section on the head, a short account of ventriculography and encephalography is also given. Useful tables of the dates of ossification and the segmental innervation of muscles are included as appendices.

The numerous illustrations and radiographs are excellent, and constitute an important feature of the book. They show judicious care in their selection and are skilfully executed. Especially helpful is the series of parallel illustrations showing surface contours in their relation to the underlying structures, and this method is also employed to aid the interpretation of difficult oblique radiographic views of the chest. The diagrams also will be most helpful to the student. Mr. A. K. Maxwell's services as artist have rendered an invaluable contribution to the work.

The arrangement of the book is good and the treatment of the subject matter is a new departure which worthily satisfies the requirements of modern anatomical teaching. The authors are to be congratulated on their important contribution to the study of "living Anatomy", and the publishers for their skill in producing this volume at such a modest price.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

February 1938

RECORDED BY
A. J. E. CAVE

PROCEEDINGS OF THE ANATOMICAL SOCIETY OF GREAT BRITAIN AND IRELAND

FEBRUARY 1938

An Ordinary Meeting of the Society was held in the Department of Anatomy, King's College, London, at 3 p.m. on Friday, 18 February 1938, the President, Prof. ALEXANDER LOW, in the Chair.

Prof. R. D. LOCKHART and Dr W. BRANDT read a paper on the "Length of striated muscle-fibres". Although Kölliker (1852) stated the fibre runs the complete length of the muscle from the tendon of origin to the tendon of insertion, and while physiological argument by Jansen (1913) and Haines (1932, 1934) is in agreement, yet descriptions and illustrations (Braus, 1921; Gray, 1935; Maximow, 1936) are given of fibres terminating and arising in tapering cones in the course of the length of a muscle, so that several individual fibres may contribute to the total length, and we understand this is still the average teaching.

Statements that the length of fibres may be "from 1 to 40 mm." (Maximow, 1936), "an inch or more" (Schäfer, 1934), "of no great length, not exceeding as a rule, 40 mm." (Gray, 1935), "not more than 15 cm. in *Sartorius*" (Cunningham, 1937), are indefinite as to whether the fibre runs from origin to insertion.

We have not yet seen the tapering method of termination and origin in the course of a muscle, described above, even under high powers of the microscope.

In an adult *Sartorius* 52 cm. long, we isolated fibres of 34 cm. and even at this length the fibre is abruptly broken in its length, and there is no sign of cone-shaped termination.

In a foetus with a *Sartorius* of 5 cm. long, we found, isolating the fibres by teasing, that they run the complete length of the muscle, so also in the case of the semitendinosus. In the adult eye muscles the fibres ran all the way.

Various specimens were on demonstration.

Prof. WHITNALL discussed fibre length in the extra-ocular muscles of reptiles and Prof. MEEK muscle fibre growth in domestic cattle. Dr MACCONAILL observed that the component fibres of ligaments appeared to be continuous structures, coextensive in length with their containing ligament. Dr FIELDING enquired whether, after muscle teasing, any syncytial arrangement of fibres had been observed: the authors replied that neither in foetal nor in post-natal muscle had they encountered any such arrangement.

Dr W. L. STRAUS (introduced by Prof. WOOLLARD) read a paper on "The embryology of muscular activity in the white rat". This paper is a preliminary report of investigations still in progress. Skeletal musculature of the rat apparently gains the ability to contract (following electrical stimulation) some time during the 16th day of prenatal life (the gestation period being 21 days)—

or at a stage of approximately 14–15 mm. C.R. length. The first responses elicited in living foetuses were flexion and extension of the upper extremity (excluding the digits), each being obtained from a definite, well-defined position in the arm. They undoubtedly were neurogenic in nature, being produced by stimulation of peripheral nerves. No responses were obtained from the lower extremity or any other part of the surface of the body. Contractions of the musculature of the lower extremity proper have not been definitely elicited until late in foetal life. The earliest movements of the hind limbs are produced by action of the trunk muscles upon the pelvis, with possible implication of the hip musculature in some instances. Dr DE BEER commended this profitable method of study and contrasted the dissimilar behavioural responses to stimulation obtained from mammalian and urodele material. Dr BARRON commented upon peculiarities of the threshold of response of foetal muscle: in the foetal of sheep myogenic responses could be elicited earlier than neurogenic responses, e.g. pupillary movements were more quickly obtained by local intra-ocular stimulation of muscle fibres than by stimulation of the cervical sympathetic. He recommended the galvanic current as the most satisfactory medium for stimulation, enquired as to details of operative technique, and asked whether a rapid onset of fatigue had been noted in the foetal rat musculature. Mr WHILLIS observed that cavitation of the developing limb diarthroses occurs in a proximo-distal sequence, an order harmonizing functionally with that of muscle response in the embryonic limb. In reply Dr STRAUS admitted that some of the movements obtained might not be truly reflex; the earliest undoubted reflex responses obtained took the form of generalized mass movements. Fatigue of foetal muscle was extremely rapid in onset, two or three stimuli being frequently quite sufficient to induce its appearance. He considered those muscles responding to the induction coil to be the physiologically more advanced: varied frequencies and the galvanic current would be adopted in further investigations. Any phase of original myogenic response (if present at all) in the white rat must be of the briefest duration.

Mr W. G. LLOYD DAVIES (introduced by Prof. WOOLLARD) read a paper on "Cleared thick sections of brain and spinal cord stained by Mulligan's method". The brain stem and spinal cord can be cut into sections of about 300μ. by a freezing microtome. The tissues themselves need not be frozen, but the base should be firmly frozen to the stage. The sections are then stained by Mulligan's technique. If a serial is not required they can all be stained together in the same dish. They are dehydrated, cleared in carbol xylol and xylol, and mounted on large slides in balsam. The advantage of the method lies in its rapidity and in the ease with which finished specimens are handled. The differentiation of grey and white matter is good, and the technique can be substituted for the more tedious Weigert method in the preparation of class material for macroscopic or low power work.

Dr S. ZUCKERMAN read a paper on the "Development of the vagina in the Green Monkey (*Cercopithecus aethiops sabeus*)". Observations made on seven immature Green Monkeys show that the vagina is not fully canalized until after the eruption of the central incisors. In the youngest specimens examined the vagina was patent only above the opening of the urethra and in the region of the fornices. The anterior fornix is a late development.

Canalization begins in the centre of the solid vaginal plate. Experiments conducted on two animals show that the solid vaginal plate can be canalized by means of oestrone. The plate is not affected by male hormone.

Dr ZUCKERMAN also read a paper on "The 'female prostate' in the Green Monkey (*Cercopithecus aethiops sabeus*)". Periurethral glands, presumably homologous with Skene's tubules, occur in many Green Monkeys. In a specimen that was injected with testosterone propionate, a male hormone, they were more numerous and more active in appearance than they are in normal control animals. Glands of identical appearance occur as antero-lateral offshoots of the part of the vagina immediately above its opening. In one anomalous specimen these glands on one side formed a solid mass which was indistinguishable from prostatic tissue. This prostatic mass extended upwards and around the posterior surface of the vagina. A glandular mass indistinguishable from prostate was also found in a Rhesus monkey (*Macaca mulatta*), and the common collecting duct of this gland also passed into the vagina. If the openings of these prostatic glands in the female monkey indicate their embryological derivation, these observations indicate that the part of the vagina from which they are derived cannot be Mullerian in origin—unless the prostate be regarded as a derivative of the Mullerian system.

The periurethral glands in the green monkey undergo squamous netaplasia under the influence of oestrogenic stimulation; comparable changes occur in the posterior urethral wall. These observations, together with those reported above, will be considered in relation to other experimental findings that bear on the development of the vagina and of the glands derived from the urogenital sinus.

Prof. W. E. LE GROS CLARK read a paper on "The endocranial cast of the Swanscombe fossil". The Swanscombe "skull", discovered by Mr MARSTON in 1935 and 1936, consists of a complete occipital and a left parietal bone. Its importance is related to its antiquity which is particularly well attested by the geological and archaeological evidence. The endocranial cast, although presenting certain features of interest, suggests that the brain of Acheulian Man had acquired the size and complexity characteristic of modern Man. Prof. WOOLLARD gave a résumé of Dr MATTHEW YOUNG's recent elaborate analysis of the Swanscombe cranial fragments, and made comparisons between this fossil and the Piltdown and London (Lloyd's) crania. Dr CAVE emphasized the importance of the Swanscombe specimen, particularly the authenticity of its geological age, in the study of human palaeontology; he welcomed the cautious interpretation of the endocranial cast. The PRESIDENT commented upon the great interest of this new discovery and upon variation in cranial and cerebral form.

Dr M. A. MACCONAILL read a paper on the "Biological significance of blood groups", based upon studies of the populations of Sheffield and Rathlin Island (Rachrai). It is well attested that the blood groups (O, A, B, AB) are genetically independent of hair and eye colour, sex, and so on. It does not follow, however, that a gene for one of these blood groups is without effect on the course of development of other characters of the individual in whom it is found. The people of Rachrai have three times as much of the B-type as is found in Sheffield people, and a smaller proportion (one-third instead of one half) of the O-type. The heterozygous combinations of hair and eye colours

show corresponding differences. The Rachrai people have six times as many instances of dark hair with blue eyes as the Sheffield people, with a converse proportion of fair hair with dark eyes. In Sheffield, the mean stature of the heterozygous classes is less than that of the homozygous classes: there is no such difference in Rachrai. A review of the post-natal development of hair and eye colour and of the blood groups suggests that the mutation forms of gene (A, B, AB) may promote the exhibition of dark hair and blue eyes in an individual who is genetically fair-haired and dark eyed. The individual correlations between blood group and colour type observed in Rachrai are completely consistent with this view. It has already been shown that hair and eye colour must be taken into account when making use of stature as an estimate of the effects of nutrition or environment. It is now suggested that the blood types of contrasted stature groups may also need to be considered in the same connexion. The numbers examined in Rachrai were: 44 for colour, 30 for blood groups, and 20 for stature. The corresponding numbers in Sheffield were 1100, 329 and 350. The differences between the two groups of people are statistically significant. The PRESIDENT congratulated the author and urged the desirability of further anthropological or biological surveys along similar lines. Prof. WOOLLARD enquired whether heterochromes manifested any other signs of biological inhibition apart from that of stature, and was informed that other physical characters, such as shoulder-width, bore out the stature findings. Prof. WOOD JONES asked whether the heterochrome growth-lag held good for both types (i.e. for both the dark-eyed, fair-haired people and the light-eyed, dark-haired people) and was informed that it did.

Dr R. J. O'CONNOR read a paper on "Experiments on the development of the amphibian nephric system". The development of the amphibian nephric system, has been studied experimentally, (*a*) by the localized destruction of various portions; (*b*) by the transplantation of tissues into its immediate vicinity. The experiments were performed at the "tail bud" stage on *Amblystoma tigrinum*, *Triton taeniatus* and *Pleurodeles waltlii*, and the results obtained have led to the following conclusions: (1) Following injury to the pronephros the pronephric duct on the same side becomes retarded in development and forms an irregular column of cells almost without a lumen. (2) This irregular column of cells reforms into a duct when the mesonephros develops, but the reformation does not occur cranial to the mesonephric region. The reformed duct now acts as the efferent duct of the mesonephros. (3) The proximity of the pronephric duct, whether it be normal or degenerate, is necessary for the development of the mesonephros. (4) The cloaca exerts little or no influence on the organization and growth of the pronephric duct, and in the union of these it is suggested that the duct plays the more active rôle. The PRESIDENT and Prof. WOOLLARD tendered congratulations upon technique and presentation. Dr MACCONAILL regarded such work as particularly illuminating and valuable in the elucidation of obscure points in embryology. Dr BRANDT complimented the author upon the excellence of his results and spoke of the important rôle of mesonephric development in the differentiation of various tissues and organs, including even the limb buds and the shoulder girdle.

Demonstrations

At this Meeting of the Society the following demonstrations were given:

(1) R. J. S. McDowall (introduced by T. Nicol): "Reversible paralysis of Deiter's nucleus."

(2) J. A. Hewitt (introduced by T. Nicol): "Sections of the central nervous system."

(3) M. Allanson (introduced by T. Nicol): "Maintenance of normal structure of the anterior pituitary in castrated male rats by ovarian grafts."

(4) T. Nicol: "Intravitam staining and the effects of hormones on the female reproductive system."

(5) W. G. Lloyd Davies (introduced by A. B. Appleton): "Cleared thick sections of brain and spinal cord stained by Mulligan's method."